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Dehydration as a risk factor for calf mortality in northern Australia

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Abstract

It is estimated that approximately one million calves die in Australia every year, with the majority unexplained. This thesis reviews losses associated with dehydration-mediated mortality during neonatal life, when there is highest risk of mortality, and reports three studies of neonatal calf dehydration.

For 12 neonatal Brahman calves at Spyglass research site (northern forest land type, Queensland), variation in hydration status was investigated (Experiment 1). Change in live-weight from birth was used as the primary measure of hydration. Half of this cohort had low live weight gain between birth and day 3 of life ($p = 0.01$) compared to calves growing at 0.95±0.24 kg/day from birth. Low growth and associated reduced plasma protein ($p = 0.04$) in 6 calves indicated reduced colostrum, milk uptake and therefore reduced body fluid replenishment compared to other calves. This positions them at high risk of mortality under adverse environmental conditions. Urea space was tested as a measure of body water; however, percentage body water estimates were unrealistically elevated (> 80% of live weight) and therefore not representative of actual calf percentage body water.

Descriptive analysis of risk factors for calf mortality was conducted for 478 and 378 tropically adapted newborn calves, respectively at Spyglass and Brian Pastures (southern forest land type, central Queensland) research sites (Experiment 2). The incidence risk of neonatal mortality in the southern forest of Queensland and northern forest of Queensland was 3.1% and 4.7%, respectively. Some risk factors and the percentage of calves exposed to high mortality risk included: dam body condition score ≤2 (8%), birth weight ≤28 kg (18%), low birth vigour (2%), large udder size (1%), at least two large teats (2%) and poor maternal protectiveness (5%). The low proportion of cows in poor body condition and good nutritional management limited the opportunity for dehydration-mediated neonatal mortality. There were no cases of prolonged heat stress around calving at the level previously associated with foetal and calf mortality. Experiment 2 indicates that low calf mortality may be achieved in any country type, provided that a low proportion of calves are exposed to high risk of mortality.

A simple mechanistic model was developed to describe milk uptake volumes and ambient temperature (heat stress) impacts on neonatal hydration. This model provided clarification for the possible risk of dehydration mediated mortality across breeding herds of northern Australia.

This thesis demonstrated variation in milk delivery, modelled that low milk delivery is associated with dehydration-mediated mortality and demonstrated that risk of mortality is low at a herd level when the risk factors associated with reduced milk delivery have low frequency.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

Muller, J.; Fordyce, G. and Anderson, A. 2016.Are neonatal calves getting enough to drink in northern Australia? Proceedings of Australian Society of Animal Production Conference. Adelaide, Australia: p 89.

Muller, J.; Brazier, N. and Campbell, J. 2016.Calf vigour at birth and neonatal mortality Proceedings of the Northern Beef Research Update Conference, Rockhampton, Australia: p 109

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No parts of this thesis were submitted for the award of another degree.

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Keywords

calf, mortality, neonatal, dehydration, hydration, risk factors, model, tropical, Australia.

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1. Thesis Introduction

Within cows that achieve pregnancy across northern Australia, median incidence of foetal and calf mortality ranges from 5% (for first lactation cows in the southern forest) to 16% (for heifers in the northern forest; McGowan *et al.*, 2014). Interquartile ranges for incidence of foetal and calf mortality were between 5% and 14% (McGowan *et al.*, 2014). Bunter *et al.* (2013)'s study focussed on tropically-adapted calf mortality and demonstrated that 67% of foetal and calf mortalities occur during neonatal life, i.e., within the week of birth.

Foetal and calf survival is important for live weight production (Fordyce *et al.*, 2016). Given the existing variation for incidence of foetal and calf survival and that a high proportion of these mortalities occur in neonatal calves, production and profit gains may be made by improving neonatal survival rate. The causes responsible for the majority of occurring neonatal mortalities in northern Australia are not defined (Bunter *et al.*, 2013, Holroyd, 1987). Although, foetal and calf mortality has been reported to be mostly associated with the nutrition, management and environment during gestation and around calving. These associations are as strong, if not stronger than associations with disease and animal factors (Bunter *et al.*, 2013, Fordyce *et al.*, 2014, McGowan *et al.*, 2014).

In various environments and cattle genotypes, nutritional inadequacy, herd management methods and environmental stress may be associated with increased incidences of cow mortality, poor dam mothering-behaviour, reduced cow lactogenesis peri-partum and low calf ability to suckle. The above risk factors may ultimately lead to reduced milk uptake by calves. Therefore the ultimate reason for many neonatal mortalities in northern Australia may be milk deprivation and dehydration.

It is hypothesised that under extensive paddock conditions, hydration status varies between suckling neonatal Brahman calves. No studies have investigated the variation in hydration status of neonatal calves in northern Australia and how hydration status may be impacted by various possible risk factors. Therefore, literature is reviewed for the specific mechanisms that are likely to ultimately result in dehydration-mediated neonatal calf mortality. Current literature is also reviewed for the behaviour of calf body water dynamics and the methods used to quantify hydration status.

An experiment aimed at quantifying variation in daily hydration status of 12 neonatal Brahman calves is reported. For 297 calving cows in a northern forest (Queensland) environment and 683 calving cows in a southern forest (Queensland) environment, the frequencies of various risk factors for calf mortality are presented for the calving season of late-2015 to early-2016. A basic mechanistic model is described, aimed at contrasting hypothetical patterns of calf milk uptake,

to estimate their hydration status through neonatal life and therefore the possible risk of dehydration mediated mortality.

The ultimate aim of research in this area is to allow the graziers of northern Australia and their advisors to identify nutritional, environmental or management related risk factors within their specific situation that can be ameliorated or eliminated to reduce negative impacts on calf mortalities, cattle herd welfare and profitability. This objective cannot be achieved without detailed and targeted research carried out in a typical extensive production system where the hypothesised risk factors to calf mortality exist.

2. Dehydration as a risk factor for calf mortality in northern Australia: A review

2.1 Introduction

Within cows that achieve pregnancy across northern Australia, median incidence of foetal and calf mortality ranges from 5% (for first lactation cows in the southern forest) to 16% (for heifers in the northern forest; McGowan *et al.*, 2014). Interquartile ranges for incidence of foetal and calf mortality were between 5% and 14% (McGowan *et al.*, 2014). The variation in incidence of foetal and calf mortality indicates that there may be scope to increase foetal and calf survival rate in many breeding herds, with consequent increases in production and profit.

In beef breeding herds of northern Australia, an increase in foetal and calf survival of 2.7% was associated with a production increase of 10 kg/cow/year (McGowan *et al.*, 2014). In economic modelling of a representative beef herd in northern Australia with 3,000 adult equivalents in which average calf weaning age was 5 months, an increase in absolute weaner production (kg/cow/year) of 8.3%, associated with an increase in weaning rate from 61 to 66%, equated to an increase in gross margin of $23 280 based on 2015 prices. This equated to a $12.23 higher margin per breeding female; therefore, if an intervention that costs less than this and achieves stated increase in weaner production, it will be profitable.

Outcome variables related to foetal and calf survival are defined as occurring within particular time-frames, including foetal and calf life (between positive pregnancy diagnosis and weaning), neonatal (within the week of birth), peri-natal (within 48 hours of birth) and post-natal (between 48 hours of life and weaning). Across mammalian species, the most critical stage of life is the neonatal period (within a week of birth), a time at which they are at high risk of inadequate milk consumption (Nowak *et al.*, 2000). Tropically-adapted beef breeding herds are no exception, as during the period from positive pregnancy diagnosis to weaning, 67% (Bunter *et al.*, 2013) or 43.8% (Holroyd, 1987) of mortality has been reported to occur during neonatal life[1]. Under frequent observations in extensively-managed Brahman and Brahman influenced herds, the majority of neonatal mortalities were unexplained (Bunter *et al.*, 2013, Holroyd, 1987).

Calf mortalities are associated with general risk factors including high temperature-humidity index during the calving period and deficiencies of energy and phosphorus in the dam. These

[1] Although neonatal calf mortality rates were not reported in Holroyd (1987), counts were reported for number of pregnancies studied (3285), total losses between pregnancy diagnosis and weaning (516), calf mortality within 48 hours of life (170) and calf mortality between hour 48 and day 7 of life (56). Therefore neonatal mortality rate was calculated as percentage of total foetal and calf mortalities that occur during the first week of life.

nutritional and environmental risk factors are much more consistently associated with calf mortalities than animal factors or any infectious disease (McGowan *et al.*, 2014). The specific causes that explain these general associations are unknown, though they may be related to the calf or cow. Nutritional or environmental stress on the cow during gestation may perturb foetal development, leading to calves that lack an ability to stand, seek teats and suckle. In addition, nutritional or environmental stress may impact on cow survival, lactogenesis peri-partum or mothering behaviour and therefore milk delivery to the calf (Fordyce *et al.*, 2014).

Neonatal calves experiencing milk deprivation in a tropical environment can lose more than 20% of body water within 1 – 3 days and some calves experiencing this level of dehydration cannot recover without assistance (Fordyce *et al.*, 2015). Based on the above general risk factors, dehydration risk and the reports that most calf mortalities occur during the neonatal stage, a highly-plausible reason for unexplained neonatal calf mortalities is dehydration due to milk deprivation. The role of dehydration in calf mortalities has not yet been defined in a paddock situation in northern Australia. Evidence is presented to support the hypothesis that neonatal dehydration is the ultimate reason explaining the foetal and calf mortality associated with nutritional and environmental stress. The mediating risk factors potentially impacting on calf dehydration are not clear in north Australian production systems and therefore literature from other regions and genotypes are discussed. The aim of this literature review is to link general associations and the possible specific mechanisms that may explain these associations. Limitations to the practical field methods used to quantify hydration status in this research are also discussed.

Without an understanding of the specific mechanisms involved, it is not possible to design economically-viable, targeted interventions to reduce calf mortality. Variation in calf hydration would indicate opportunity to develop interventions aimed at decreasing the frequency of calf dehydration and therefore the incidence of calf mortality.

2.2 General risk factors influencing calf survival

Peak rainfall and temperature in northern Australia generally occurs from October – March and October – January, respectively, with a tendency for later peaks further north (Bureau of Meteorology, 2016a). Following rainfall patterns, pasture protein and digestible dry matter, as percentages of dry matter generally peak between December and January (Coates, 2000, Holroyd *et al.*, 1977, Norman, 1963, Robinson and Sageman, 1967, McIvor, 1981, Squires and Siebert, 1983). Cows have increased chance of conception during the peak of pasture quality, and therefore conceive naturally or may be mated around this time to synchronise the subsequent lactation with better pasture quality and avoid lactation during the dry season. Therefore the majority of cows in northern Australia are experiencing dry season conditions and low quality pasture during mid-late

gestation. Consequently, the majority of calves in northern Australia are born between the months of October and January, with a tendency for later peak of calving further north (Bortolussi *et al.*, 2005).

Within pregnant topically-adapted cows, a 4% increase in foetal and calf mortality was associated with a crude protein:dry matter digestibility ratio of <0.125 during the dry season, compared to a ratio ≥0.125 during the same period (McGowan *et al.*, 2014). Stalker *et al.* (2006) reported that in cows fed supplemental protein during late gestation maintained body weight and those not supplemented lost 29 kg, body condition prior to calving differed between groups (5.1 vs. 4.7 on a 9-point scale). Low cow nutrition was associated with a 5% decrease in foetal and calf survival (Stalker *et al.*, 2006). Vargas *et al.* (1999) reported that within Brahman cows of third or greater parity, calf survival from birth to weaning was 88.8% ± 10.76% and 94.1% ± 4.74%, respectively for cows in "thin or worse condition" or with some fat cover. Cows of body condition score <2 (5-point scale) during pregnancy and with phosphosus:metabolisable energy status (mg P/MJ ME) of <500 for the subsequent wet season pasture, foetal and calf mortality was eight percentage points higher than those cows of body condition score ≥4 and with P/MJ ME ratio of ≥ 500 during the respective periods (McGowan *et al.*, 2014). These results of McGowan *et al.* (2014b) indicate that absolute and relative levels of nutrients in the dam diet are important and that protein or phosphorus may be a limiting nutrient when energy levels are higher. In an extensively-managed herd of 7.3±1.5-years-old Brazilian Nellore (*Bos indicus*) cows, those that had body condition score >3.5 and ≤3.5 at parturition were ranked to have higher and lower body condition score at 165 days pre-partum (Ayres *et al.*, 2014). McGowan *et al.* (2014b) measured body condition at a median stage of approximately 130 days pre-partum. Given the carry-over effect of body condition during gestation, and that the above association of McGowan *et al.* (2014b) is based on pasture phosphorus data around the calving period and not during gestation suggests that impacts of cow body condition and phosphorus status on foetal and calf mortality may be mediated by nutritional status around calving and lactation yields, rather nutrition available to cow during foetal development. Similarly, dietary pasture protein for cows was measured by McGowan *et al.* (2014b) during gestation, and therefore may also have implications for level of body reserves around parturition. The situation in tropically-adapted primiparous heifers may differ from cows, as level of nutrition in the first two trimesters of gestation did not impact on heifer body condition at parturition (Sullivan *et al.*, 2009c).

Cows with hip height >140 cm had a foetal and calf mortality of four percentage points higher than cows of hip height <125 cm (McGowan *et al.*, 2014).Foetal and calf mortality was associated with height independently of genotype (McGowan et al., 2014b. This is consistent with the Green *et al*. (1991) study on feedlot cows fed to maintain live-weight, where Brahman crossbred

cows had a hip-height of approximately 4 cm higher than Sahiwal crossbred cows. From day 48-174 post-partum, the Brahman crossbred and Sahiwal crossbred cows had maintenance requirements of 27.7 and 25.4 Mcal/cow/day (Green *et al.*, 1991). Therefore the higher maintenance requirement of the Brahman crossbred cows may be related to their higher hip-height and therefore frame-size. Assuming maintenance requirement is explained by hip-height, if all animals were on a similar level of nutrition, for example in a paddock situation, then taller cows may have available less ME to direct to foetal development and lactation, which may explain the higher risk of foetal and calf mortality in tall cows.

For herds of northern Australia, the specific risks for deficiencies of energy, protein and phosphorus during gestation and early lactation and their effects on calf mortality are not clear. Clarification requires further study involving measurement of calf mortality, its risk factors and the mediating effects on foetal development and lactation. Although the study ofMcGowan *et al.* (2014) outlines incidence of foetal and calf mortality and associated risk factors for commercial breeding herds across northern Australia, it does not provide specific information on the timing of foetal and calf loss. If the specific mediating risk factors are clarified, this could have far reaching benefits across northern Australia. Studies in other genotypes and environments describe the possible specific mechanisms involved that may explain the described general associations of McGowan *et al*. (2013).

In the study of Corah *et al.* (1975), second-lactation *Bos taurus* cows were on restricted nutrition during days 100 to 70 pre-partum and subsequently were either continually restricted, or supplemented until calving. No-supplementation was associated with a 54.8 kg weight loss during the study period. Within the non-supplemented group, 10% of calves were born dead and 19% of the calves died prior to weaning due to scours. In the supplemented group, no calves had died within a month of birth. Corah *et al.* (1975) did not describe whether the scours were nutritional or infectious in nature. The supplemented cows and the non-supplemented cows had milk production of 5.5 and 4.1 kg/day, although timing and frequency of weigh-suckle-weigh measures used to calculate daily milk production were not reported. Compared to the supplemented cows, non-supplemented cows gave birth to calves that were 3.7 kg lighter, and were reported to be slower to suckle (Corah *et al.*, 1975). It is difficult to identify specific reasons for scour related mortality in the above study, though a possibility is that low colostrum production or low colostrum uptake by calves and therefore low immunoglobulins available for the calves may have created a risk for infectious diarrhoea and therefore mortality. The risk of infectious diarrhoea is likely not as high in northern Australia, though colostrum or milk deprivation is likely to lead to dehydration mediated calf mortality (Fordyce *et al.*, 2015).

Three consecutive pre-partum nutrition experiments were conducted on *Bos indicus* crossbred cows at a site in the dry tropics of northern Australia during 1986, 1987 and 1988 (Fordyce *et al.*, 1997). The method of each experiment involved comparing reproductive outcomes of cows that were and were not supplemented (energy and protein) for 42-54 days during mid-late pregnancy. Foetal and calf survival rate was not associated with supplementation in any of these experiments. Interestingly, in the year of poorest dry-season pasture conditions, 1987, supplementation was associated with reduced rate of weight loss (-0.63 kg/day vs. -1.25 kg/day), though not sufficiently reduced to significantly decrease live weight by cessation of supplementation (409 vs. 382 kg). During the supplementation period of 1987 non-supplemented and supplemented cows lost 0.9 and 0.5 of a condition score, as measured on a 9-point scale. Although there was a tendency for increased foetal and calf survival due to supplementation in a season of poor pasture conditions, e.g. where non-supplemented and supplemented cattle had foetal and calf survival of 96% (n=25) and 80% (n=25) (Fordyce *et al.*, 1997), it is not clear whether higher nutritional stress would have been associated with significant level of foetal and calf mortality. This tendency may lead to significant differences when there are higher reductions in live weight as occurred in Corah *et al.* (1975). During 1986, supplementation was associated with decrease in rate of weight loss (-0.35 kg/day vs. -0.54 kg/day) but not increased weight at end of supplementation (332-338 kg vs. 320 kg). During 1988, which had good pasture conditions, live weight and cow body condition remained high throughout the supplementation period with no change in live weight or body condition due to supplementation. There was no effect of supplementation on lactation yield, although this was measured only once for each cow within experiment, when calves ranged in age from 8 and 67 days of age. Therefore, though this provides some indication of overall lactation, it does not account for the increase in lactation yields that occur during the first week post-partum (see section 2.3.2) and effects of nutrition on early lactation yields may not be readily expressing using this method. Overall, the impacts of immediate pre-partum supplementation appear not to be associated with foetal and calf survival, though overall nutritional management throughout gestation may have more profound impacts.

Average weight of weaners in a study of low- and high-nutrition cow groups were 161 ± 4 kg and 148 ± 4 kg, respectively Corah *et al.* (1975). Compared to cows fed no supplement, pregnant cows fed 0.45 kg supplement/cow/day (42% CP) for 277 days pre-partum had increased weaning rate regardless of whether cows were on a post-partum diet of meadow pasture (97% vs. 92%, respectively) or hay (100% vs 90%, respectively; Stalker *et al.*, 2006). The cows of Stalker et al. (2006) fed supplement also had calves of increased average daily gain to weaning, for cows on post-partum diet of meadow pasture (0.97 vs. 0.92 kg/d, respectively) and hay (0.93 vs. 0.90 kg/d, respectively). Those cows fed supplement maintained live weight during the pre-partum period,

though cows not fed supplement lost 29 kg. Cows fed supplement had improved body condition score at calving compared to cows not fed supplement (Stalker *et al.*, 2006). In the studies of Corah *et al*. (1975) and Stalker *et al.* (2006), given that improved pre-partum nutrition was associated with both increased weaner survival and average weaner weight, and that weaner production (kg/cow) is a function of weaner survival and growth, this also indicates an impact of nutrition on total weaner production (kg/cow).

Both Corah *et al*. (1975) and Stalker *et al.* (2006) describe that cow groups differing in calf mortality rate also differ in change in live weight and body condition during gestation. Given that gestation coincides with the dry season for many breeding herds in northern Australia, this suggests research focussed on identifying the causes involved in these associations may be warranted. However, this is not consistent with McGowan *et al*. (2014), where cows calving out of season did not have increase rate of foetal and calf mortality. The reason for the finding of McGowan *et al*. (2014) may be that cows calving out-of-season may gain body reserves and live weight when gestation coincides with the wet season, though lactation in the dry season has consequences as it extends post-partum anoestrous and thus limits cow lifetime weaner production.

In a comparison of cows that were lactating and non-lactating prior to mating, those that were lactating exhibited a 3.6% higher rate of foetal and calf survival (McGowan *et al.*, 2013). For this association, non-lactation was defined as being a heifer, or not achieving pregnancy or aborting. When heifers were excluded from analysis, the association was 7.6%. This is consistent with a herd of 445 Droughtmaster cows, where those that lactated in the previous season had a 10% higher calf survival rate than those that did not lactate in the previous season (Hetzel *et al.*, 1989). Given the low repeatability of foetal and calf mortality as reported in above studies, physical abnormalities, e.g. of the uterine or udder anatomy, are occurring at low frequency. A low frequency of physical abnormalities may explain why nutritional deficiency and environmental stress is consistently impacting on foetal and calf loss from year to year.

Increased foetal and calf mortality was associated with general measures of environmental stress and dam nutritional inadequacy. Temperature-humidity index >79 for >14 days consecutive around calving was associated with a four to seven percent increase in foetal and calf mortality, depending on land type (McGowan *et al.*, 2014). The specific reasons explaining the above associations are not clear. There is a paucity of research on the nutritional and environmental reasons that may impact on calf mortality, especially during the neonatal period. Further clarification required focussed study on how these risk factors impact on foetal development, early lactogenesis and mothering behaviour. With further understanding of specific mechanisms acting on calf mortality, interventions may be designed that are biologically-targeted and economically-viable in increasing calf survival rates. Studies focussed on the effects of nutrition and environment

on calf vigour, cow milk production and mothering behaviour in northern Australia are limited; therefore, studies from other regions are reviewed in the following sections (2.3 to 2.3.4).

2.3 Milk availability for calf

A likely specific reason for the association between foetal and calf mortality and nutritional and environmental stress is lack of calf milk uptake and dehydration within a week of birth. In this section, studies are discussed describing how impacts of nutrition and environment may impact on reduced calf milk uptake, which may be mediated by cow mortality, low milk production, failure of the dam to behave in a manner that facilitates calf access to milk, or low calf ability to suckle.

2.3.1 Cow mortality

In Brahman cross cows in drought conditions, lactating cows had a higher incidence of mortality (0.42) than non-lactating cows (0.22, Fordyce *et al.*, 1990). All mortalities of lactating cows were associated with calf mortality (Fordyce *et al.*, 1990). The primary driver of cow mortality is poor body condition, whether in drought conditions (Fordyce *et al.*, 1990) or good seasons (Mayer *et al.*, 2012). Poor condition cows had higher incidence of mortality (0.51) and fat cows had a lower incidence of mortality (0.03), than moderate condition cows (0.21). Cow mortality rate is also a function of dry season weight loss (Mayer *et al.*, 2012).

In a mixed herd of *Bos indicus and Bos taurus* cows, incidence of mortality was greater in 8-10 year olds (0.023) than younger cows (0.05 – 0.015, Frisch, 1973). In Brahman cross cows under drought conditions, cows ≥8 years old had a higher incidence of mortality (0.37) compared to cows < 8 years old (0.24), despite no difference in body condition between these groups (Fordyce *et al.*, 1990). Similarly, dam age and body condition were reported to interact in their effect on cow mortality (Mayer *et al.*, 2012), where older cows were more susceptible to mortality when in poor condition, compared to younger cows. This may be explained by older cows having less ability to convert available pasture into useable nutrients.

Pasture conditions clearly have implications for cow body condition and weight change. In the study of Fordyce et al., (1990), most cow mortalities during the drought occurred in November, though mortalities had also occurred in late October, which coincided with decline in pasture quality and little decline in pasture quantity (Fordyce *et al.*, 1990).

The high risk of cow mortality and associated calf mortality may be due to nutritional deficiencies that are also limiting lactation, especially when cows are losing weight or body condition.

2.3.2 Cow milk production

Lactogenesis has been described to consist of two phases, where the first phase is characterised by low-levels of mammary epithelium differentiation and milk synthesis during the third trimester of gestation. The second phase involves the completion of differentiation and initiation of ample milk synthesis during the days pre-partum (Hartmann, 1973, Fleet *et al.*, 1975). In dairy cows, two quarters were milked at least daily, from the day of calving until 3 days post-partum, where milk yield increased from 2.9 ± 0.3 to 8.6 ± 1.4 L/d (Hartmann, 1973). In the same cows, the other 2 quarters were milked daily throughout late gestation, and milk yield increased rapidly from about 2-3 days before parturition until 2-3 days after parturition, with increase from <1 kg/d to approximately 12 kg/d during this period (Hartmann, 1973). In four pregnant first-lactation Friesian cows milked through pregnancy, milk yield dropped to minimum volumes at 1-20 days prior to parturition and then gradually increased. Minimum milk yield varied between the cows, and occurred at 20, 10, 9 and 1 day pre-partum (Wheelock *et al.*, 1965). Milk production increased rapidly between parturition and 5 days post-partum, with milk yield approximately tripling within this time (Wheelock *et al.*, 1965). If tropically-adapted cows do not reach daily milk yields that meet neonatal calf fluid requirements, especially in the first 2-3 days of calf life, there is high risk of dehydration-mediated calf mortality (Fordyce *et al.*, 2015). Specific studies focussed on initiation of lactation and daily milk yields for within the first week post-partum are limited in extensively-managed beef cows.

In Brazilian milking Gir (Bos indicus, L.Ledic *et al.*, 2002) and *Bos indicus*-Holstein crossbreds (Glória *et al.*, 2012), rates of increase in daily milk production between onset of lactation and peak daily milk production (23 to 36 ± 22 days post-partum) were 0.05 kg/d and 0.22 ± 0.18 kg/d. In Indian Sahiwal, average rate of increase in daily milk production between week 1 and 5 of lactation was approximately 0.05 kg/d (Rao and Sundaresan, 1979). Although *Bos indicus* cows increase in daily milk yield over the first month of lactation, the lactation of *Bos indicus* cows may only be initiated 2-3 days pre-partum, with greatest rate of increase in lactation up to 2-3 days post-partum, as reported in studies on *Bos taurus* cows.

For 2034 lactations, as measured in 681 Indian Sahiwal cows, average daily milk production during the first week of lactation was approximately 6.7 kg/day in summer and 8.5 kg/d in winter. In winter, heat stress was lower and fodder quality was higher (Rao and Sundaresan, 1979). This is consistent with Rao and Sundaresan (1979) describing low repeatability and heritability of early lactation yield measures, of approximately 0.2. In *Bos indicus*-Holstein crossbred cows, those calving before and after the wet season had early lactation yields of 7.51 ± 0.31 kg/d and 8.90 ± 0.26, respectively (Glória *et al.*, 2012). This is consistent with modelled lactation curves for Holstein-Gir crossbred cows calving in the dry and wet season, where the predicted intercept

parameter representing yield at the start of lactation was 2.58 and 2.34 kg/d, respectively (Madalena *et al.*, 1979). In N'dama cows under village management, a similar effect of nutrition was reported, where cows that gestated throughout the dry season and those that experienced wet season conditions during 5-6 months pre-partum produced 75 (n=31) kg and 95 kg (n=132) milk during the first two months of lactation, respectively (Agyemang *et al.*, 1988). Body condition was reported to be higher in cows that experienced rainfall in gestation, though was not quantified (Agyemang *et al.*, 1988). There may be a weakness in this study, given that calves were allowed to suckle after hand milking these animals, although both groups were hand-milked using a consistent method. Pasture growth from rainfall events coincided with peaks in milk production and calf growth (Agyemang *et al.*, 1988). In Kenana (*Bos indicus*) cows, annual milk yield was not impacted by season of calving, but was impacted by year, which was attributed to differences in management practices including supplementation (Wilson *et al.*, 1987). Given the importance of cow pre-partum nutrition to early milk yields across genotypes and environments, and that high milk production does not occur until 2-3 days peri-partum, low nutritional status in cows may lead to daily milk yields that do not reach calf fluid requirements until after > 2 days post-partum.

Based on a series of dairy studies focussed on milk production between 8 and 13 weeks post-partum, each additional 11.6 MJ ME/d consumed by cows was estimated to increase milk production by 0.9 kg/d (Coulon and Remon, 1991). There are limited studies focussed on impacts of nutrition on beef cattle early-lactation yields and neonatal growth, especially under field conditions. During the first lactation of *Bos taurus* beef heifers, efficiencies at which ME is converted to lactation energy and tissue energy was reported to be similar to dairy cows, despite the lower milk yields of beef cows (Freetly *et al.*, 2005).

In dairy cows and heifers experiencing their first 8 weeks of lactation, milk yield is reportedly higher with additional dietary protein (Cowan *et al.*, 1980). There was an interaction effect, where cows fed a diet of 40:60 hay:concentrate had higher protein intake than those on a 60:40 diet. The higher level of milk production in cows consuming more protein (and roughage) was evident within the first week of lactation (Cowan *et al.*, 1980). Along with energy and protein, phosphorus is also a constituent of milk. A low phosphorus diet (0.08 P/kg dry matter compared to 0.28 g P/kg dry matter) during early lactation was associated with an average decrease in early lactation milk production of 2.2 – 2.9 L/d (Castells *et al.*, 2014).

Milk production may also be, to a lesser extent, impacted by previous diet and therefore tissue reserves (Lalman *et al.*, 2000). In dairy cows, during the negative energy balance experienced during early lactation, an average of 10% to 15% of energy output in milk was derived from the mobilisation of body reserves (Wood *et al.*, 1980). For dairy cows in early lactation, the conversion efficiency of energy within body reserves to milk energy was 82-84% (Moe *et al.*,

1971). A similar conversion rate of tissue energy to lactation energy of 88% was demonstrated in first-lactation *Bos taurus* beef females (Freetly *et al.*, 2005). With similar conversion rates across genotypes, similar conversion efficiencies may also occur in *Bos indicus* females. This is supported by the finding that Brahman cow daily lactation yield tended to decrease by an average of 1 kg for each unit decrease in cow body condition score (1 – 5 scale, McBryde *et al.*, 2013).

For Friesian cows, Bell *et al.* (2000) calculated that between parturition and seven days post-partum, protein balance rapidly decreased from approximately -150 g/day to a nadir of -600 g/day. For late-gestation ewes on energy-sufficient diets a deficiency of dietary protein exacerbated protein mobilisation from body reserves and reduced the deposition of protein within mammary tissues (Bell *et al.*, 2000, McNeill *et al.*, 1996). Therefore protein deficiency may also have implications for early lactation milk yields in extensively-managed beef cattle. The above studies support the view that only when energy is adequate, and that protein is a limiting nutrient will increases in dietary protein improve milk yields. In dairy cows, depletion of protein reserves has been associated with decreasing milk production until dietary protein supplied almost all protein required for maintenance and production, where during 8 weeks of depletion, milk production declined by 13.3% and body weight declined by 12.9% (Botts *et al.*, 1979). Based on a regression of Friesian cow nitrogen balance and week of lactation, it was calculated that protein mobilisation from body stores provided a sufficient amount of protein for 4.5 kg milk per day, assuming no deficiency in other nutrient precursors for milk (Cowan *et al.*, 1980). In tropically-adapted first-lactation cows, a low phosphorus diet (0.08, compared to 0.22 g P/kg) during late gestation was associated with an average decrease in early-lactation milk production of 1.6 L/d (Castells *et al.*, 2014). Therefore phosphorus as a precursor for milk may be stored and may buffer deficiencies during lactation to some extent.

The extent that negative energy and protein balance occurs in extensively managed beef breeding herds during early lactation is poorly understood. The principle that limited dietary intake or body reserves limits milk production is likely to apply to extensively managed beef breeding herds as it does to dairy herds. Although, given the lower level of milk production in beef herds, a lower level of nutrition and body reserves would be required.

In Brahman cows, there is an apparently-linear decline in milk yield as ambient temperature increases from 35^{o}C to 43^{o}C (Brody, 1956). This increase in temperature appeared to explain a drop in milk production of approximately 1 kg/day. In dairy cows, ambient temperature has a two-day lag effect on daily milk yield, where an increase in black globe temperatures from 25^{o}C to 35^{o}C appeared to be associated with a decrease in daily milk yield of approximately 2.4 L (Collier *et al.*, 1981) and where change in milk yield (kg) per increase in temperature-humidity index unit was -0.88 for Holsteins and -0.6 for Jerseys (West *et al.*, 2003). In dairy cows, increased heat stress

during late gestation has been associated with reduced mammary cell proliferation, as measured 20 days prior to calving which, in turn, reduced subsequent average daily milk production (Tao *et al.*, 2011). An increase in foetal and calf mortality is associated with high temperature-humidity index for >14 days in the month of calving (McGowan *et al.*, 2014). A possible reason for this association may be that cow milk production may be reduced to levels that mediate neonatal dehydration and mortality if cows experience >14 days of heat stress.

As explained above, for various environments other than northern Australia, lactation has been shown not to be initiated until within the week pre-partum and has been shown associated with pasture condition, precursor nutrients to milk in diet and body stores (including energy, protein, phosphorus) and prolonged high temperature-humidity index. At the end of the wet season across northern Australia (around March), pasture generally declines in percentage digestibility (McIvor, 1981, Squires and Siebert, 1983) and percentage crude protein (Coates, 2000, Holroyd *et al.*, 1977, Norman, 1963, Robinson and Sageman, 1967). Crude protein of pasture falls during the dry season (3% in May to 1.5% in October-November (Adrnt and Norman 1959). Given the above, it is likely for many breeding herds in northern Australia that late-pregnancy coincides with decreasing pasture and therefore diet quality and increasing temperature-humidity index. Consequently, this is a period when breeding cows are usually losing live-weight. Given that lactogenesis does not occur until the days peri-partum (Hartmann, 1973, Fleet *et al.*, 1975), the above nutritional and environmental risk factors potentially also reduce lactation yields in the first week post-partum to levels that results in dehydration mediated calf mortality.

If the discussed associations and their importance in driving calf hydration and survival are quantified with future research, this may allow opportunity to better design management interventions that are targeted and reducing risk factors for low milk production in a strategic and economically-viable way.

In humans, there is a 31% increase in milk production one week into second lactation compared to first lactation (Moritz, 2013). There is no difference in milk production between first- and second-lactation mothers at 4 weeks into lactation (Ingram *et al.*, 2001). Therefore, if a similar relationship exists in cows, then primiparous cows are delivering reduced milk to neonates compared to higher parity cows. This may at least partially explain a higher incidence of neonatal mortality in primiparous cows, compared to higher parity cows (Bunter *et al.*, 2013).

Calves of cows with pendulous udders had reduced level of passive transfer, indicating reduced colostrum consumption (Boyd and Hogg, 1981). Therefore studies on milk uptake of calves should account for poor udder conformation.

2.3.3 Calf ability to suckle

In Brahman calves, poor birth vigour has been associated with neonatal mortality (Muller 2007). This is consistent with the report of Kim *et al.* (1988), where Brahman calves without an instinct or ability to suckle took 409 minutes from birth until suckling, while other Brahman calves suckled within 227 minutes of birth. In Friesian calves bottle-fed their first colostrum *ad-libitum*, uptake volume was associated with calf vigour, as defined by attempting to stand within the first hour of life (Vasseur *et al.*, 2009). It is critical that the calf is able to access milk within the first days of life for adequate hydration (Fordyce *et al.*, 2015) and passive immunity protection (Weaver *et al.*, 2000). Calf ability to suckle may be impeded by an inability to stand, or by an apparent insensitivity to teats during teat-seeking behaviour (Kim *et al.*, 1988). Kim *et al.* (1988)'s report is consistent with calf autopsies where hairballs have been found in the calf abomasum, derived from hair around the udder or from the calf licking itself (Bellows *et al.*, 1987). In studies on *Bos indicus* calves (n > 3000), frequency of low vigour has been reported at 4.75% in Brazilian Nellore (Schmidek *et al.*, 2013) and 10.6% in a mixed herd of Brahman and Brahman-influenced calves (Riley *et al.*, 2004), with other studies reporting relative frequencies of weak Brahman calves (n > 3000) within this range of values (Riley *et al.*, 2004, Wythe, 1970). As birth vigour heritability values ranged from 0.01 to 0.09 in Nellore calves (Schmidek *et al.*, 2013) and was 0.09 in Brahmans, management interventions may provide greater improvements in calf vigour than selection (Riley *et al.*, 2004). A delay from birth to first suckling has been reported to vary with season of calving (Edwards, 1982), which is likely a function of nutritional and environmental conditions experienced by the dams. This is consistent with a study on Brahman calves where the relative frequencies of low-vigour calves differed between months of birth (Wythe, 1970). The following discussion outlines how calf birth vigour may be affected by the nutrition and environmental experienced by the dam during pregnancy, and how nutritional or environmental effects on calf vigour may be at least partially mediated by maternal-foetal hormones or birth weight.

Some neonates are born within the normal range for birth weight, yet have limited ability to suckle. This may be explained by developmental programming that can occur independent of birth weight (Barker, 2004). This was demonstrated in a herd of Brahman cows where there was no difference between birth weights for those calves that lacked an ability or instinct to suckle and normal calves(Kim *et al.*, 1988). However, calves at either extreme of birth weight are at higher risk of low vigour (Kim *et al.*, 1988) and peri-natal mortality (Frisch, 1973, Hoffman *et al.*, 1977).

In Friesian calves bottle-fed their first colostrum *ad-libitum*, uptake volume was correlated with birth weight ($r^2 = 0.37$), as defined by attempting to stand within the first hour of life (Vasseur *et al.*, 2009). This was under controlled yard conditions, where the calf was held in the corner and

bottle fed and it is unclear how calf birth weight and vigour may be associated with neonatal milk uptake in extensively-managed beef herds. In Friesian calves, birth vigour and birth weight were included in the same model, suggesting that birth weight and calf vigour are not completely related in their impact on calf colostrum and milk consumption in early neonatal life (Vasseur *et al.*, 2009).

In Brahman calves, compared to calves of moderate birth weight, those of low birth weight were at higher risk of mortality (Reynolds et al 1980). This is consistent with a study where in a dry-tropical environment, Brahman calves that died during neonatal life were on average 6.1 kg lighter than those that survived (Muller, 2007). The low birth weight calves of Muller (2007) did not differ in height from moderate-birth-weight calves and therefore the low weights were due to lack of soft tissue. In a humid tropical environment, compared to reference Nellore (*Bos indicus*) male and female calves of 29-34 kg and 27-31 kg at birth, respectively, those <24 and <22 kg at birth had higher odds of low birth vigour (3.9±0.3) and mortality within the first month of life (2.8±0.2; Schmidek *et al.*, 2013). Therefore, the association between decreased birth vigour and increased calf mortality may be at least partly explained by low birth weight. In extensive beef breeding herds, the specific risk factors affecting foetal development to ultimately affect newborn vigour are poorly understood. In infants, low birth weight can be associated with physiological immaturity (Phalen *et al.*, 2012) and the lower the birth weight, the higher the risk of issues including neurodevelopmental delays (Mercier *et al.*, 2010). Some risk of neurological issues in infants may be alleviated by higher milk uptake (Vohr *et al.*, 2006).

Low birth weight may also have implications for rates of dehydration. Calves are known to lose body water more rapidly in hot conditions (Fordyce *et al.*, 2015), though this study was conducted on eight calves within one environment and the water loss for a variety of environmental conditions and birth weights could not be quantified. In tropical and subtropical environments, an interaction may exist where low birth weight calves may be more susceptible to high temperature and humidity, due to increased surface area:weight ratio and therefore increased relative loss of body water loss to dissipate excessive body heat. This is consistent with the inverse relationship between infant body weight and rate of insensible body water loss (Wu and Hodgman, 1974). In pre-mature infants that were exposed to thermoneutral conditions and an ambient temperature of ~35^{o}, the increase in insensible water loss was higher in those of body weight <1.25 kg (73%) than those of body weight of 1.75 – 2.25 kg (46%; Bell *et al.*, 1980). Specific body water dynamics during dehydration are discussed in detail in section 2.5.

In first-lactation *Bos taurus* cows, foeto-pelvic disproportion and dystocia is primarily explained by high birth weight and to a lesser extent, by low pelvic area (Hickson *et al.*, 2008, Naazie *et al.*, 1989). Holroyd (1987) reported a trend of decreasing relative frequency of dystocia in cows of increasing *Bos indicus* content. This is further supported by a study demonstrating that

Brahman and Hereford cows had average birth weights of 30.1 and 33 kg (Roberson *et al.*, 1986). Secondly, compared to Charolais cows with a Charolais foetus, Brahman cows with a Brahman foetus had lower uterine artery and umbilical blood flow rates, and foetal weight at day 227 ± 4 of pregnancy (Ferrell, 1965). This is consistent with a study where cows of Brahman-influenced genotypes had calves of the lowest birth weight, the lowest ratio of calf birth weight to cow pelvis size and lowest relative frequency of dystocia compared to cows of various *Bos taurus* genotypes (Morrison *et al.*, 1989). Even if only first-calving cows (i.e. those at highest risk of dystocia) are considered, it is likely that *Bos indicus* cows are at lower risk of dystocia than *Bos taurus*, given that *Bos indicus* heifers need to reach a higher live-weight to attain puberty (Rodrigues *et al.*, 2002) and pelvic area is associated with live weight (Gaines *et al.*, 1993, Norman, 2002). In addition, *Bos indicus* herds are generally first mated at a later age than *Bos taurus* herds, for example, first mating at two years of age instead of one. Therefore, in *Bos indicus*-influenced breeding herds, and especially those of pure *Bos indicus*, the role of birth weight in explaining calf vigour (Riley *et al.*, 2004, Kim *et al.*, 1988) is more likely due to low birth weight than high birth weight.

In *Bos taurus* heifers, the birth of weak calves only occurred when they were on a diet of low protein content for >3 weeks during late pregnancy (Bull *et al.*, 1974). For heifers consuming approximately 0.45 kg crude protein/day in late pregnancy, the frequency of weak calves was 34% (n=50, Bull *et al.*, 1974). The frequencies of weak calves were 15 and 0% for beef cows on diets with respective crude protein contents of 0.45 kg/day and 0.91 kg/day during the last 60 days of gestation (Bull *et al.*, 1974). Birth weight differences were not reported. The higher relative frequency of weak calf births in heifers on 0.45 kg crude protein/day than cows on 0.45 kg crude protein/day may reflect the higher protein requirement of heifers and indicates that growing pregnant heifers may need additional nutritional support in order to avoid the birth of weak calves.

Birth weight can be impacted by foetal growth rate (Vonnahme, 2012) and gestation length (Reynolds *et al.*). Foetal growth is dependent on a number of factors including blood flow to the placenta, placenta vascular development (Reynolds *et al.*, 2006). Blood flow to the placenta is associated with rate of nutrient delivery to the foetus and rates of placental secretion of hormones, growth factors and cytokines (Robinson *et al.*, 1995). The effects of dam nutrition and heat stress on foetal development are discussed below.

Bos indicus-Bos taurus composite heifers on high and low levels of nutrition during the first trimester had foetal crown-rump lengths of 1.8±0.05 and 1.6±0.05 at day 39 of gestation, respectively (Micke *et al.*, 2010). *Bos indicus-Bos taurus* composite heifers of the low-high and high-high nutrition during trimesters one and two had calves that did not differ in birth weight, though they were on average 3 and 2 kg heavier than heifers of the low-low and high-low groups (Micke *et al.*, 2010). Despite the effects of nutritional treatments that occurred throughout the first

trimester of pregnancy (Micke *et al.*, 2010), Camacho (2013) reported that during the period of feeding Simmental cows either adequate or deficient diets between days 30 and 140 of pregnancy, rate of blood flow to the gravid horn of the uterus did not differ. Although, all cows of Camacho (2013) were on adequate nutrition between days 140 and 198 of pregnancy (i.e., second trimester). During this period, there was an approximate 2 L/min increase in blood flow to the gravid horn of the uterus for cows on restricted nutrition earlier in pregnancy, compared to cows on adequate nutrition throughout the experiment. In the same study, nutrient restriction in the first trimester was associated with a linear decrease in body condition, where lower body condition remained into late gestation, compared to control cows (Camacho, 2013). Nutrition of the dam in late gestation has been shown to impact on birth weight, where calves for which late gestation coincided with pasture growth, were on average 1 kg heavier than calves born in the dry season (Fordyce *et al.*, 1993). Similarly, in *Bos taurus* cows, compared to cows on high energy during late gestation, cows fed low energy diets had reduced calf birth weights, higher incidence of neonatal mortality and decreased weaning weights of calves without any increase in calving difficulty (Corah *et al.*, 1975). The above studies indicate that birth weight is impacted by nutrition throughout gestation, where improved nutrition in the first trimester may provide some positive growth responses, though the greatest impacts of nutrition appear to occur during the second trimester. The apparent increase in efficiency during the second trimester of pregnancy may be a compensatory mechanism that may overcompensate if the cow is on poor nutrition in the first trimester, followed by improved nutrition in the second trimester. This is not surprising given the high rate of foetal growth during this period compared to the first and third trimesters (Eley *et al.*, 1978). Due to seasonal rainfall patterns and therefore pasture quality (Norman, 1963, Robinson and Sageman, 1967, Holroyd *et al.*, 1977, McIvor, 1981, Squires and Siebert, 1983, Coates, 2000) and herd management across northern Australia (Bortolussi *et al.*, 2005), many breeding herds are likely experiencing decreasing pasture quality as their pregnancies progress. Therefore, many breeding herds may be at high risk of nutritional deficiency during mid- and especially late-gestation and consequently at risk of producing low birth weight calves.

In calves that were induced to be born at 277 days of gestation (approximately 2 weeks pre-mature), heart rate and respiration rate remained high through neonatal life, while other measures within normal ranges included rectal temperature, blood gases and metabolic traits, although these calves only grew 2.2±0.3 kg in this period (Bittrich *et al.*, 2002). Given the relatively low growth, underdeveloped calves may not have survived under more stressful circumstances, even if apparently vigorous at birth. Respiratory rate and heart rate has been shown to decrease over neonatal life, in calves born naturally (Piccione *et al.*, 2010). The potential for heart and respiration rate to have been compensating for some problem in the pre-mature calves may explain similar

issues in low birth-weight calves. The reasons for poor suckling ability in normal-birth-weight calves is poorly understood, but may relate to perturbations in organs or systems critical to vitality and homeostasis in the newborn. For example, lambs that took longer to stand and suckle after birth had diminished thermoregulatory capacity (Dwyer and Morgan, 2006).

The association between low-birth weight and low birth-vigour (Schmidek *et al.*, 2013) is suggestive that poor overall foetal growth may be associated with a reduced capacity of organ systems in the newborn calf. In humans, maternal diet plays an important role in the epigenetic programming of the foetus, which persists through the life of the offspring (Barker, 2004). Poor maternal diet in humans is associated with foetal programming that leads to negative consequences for the offspring including increased vulnerability adverse environmental conditions and increased risk of disease (Barker, 2004).

The effects of insults on the foetus (e.g., poor maternal nutrition) on foetal programming may not always be detectable by a reduction of birth weight (Harding, 2001). Individuals of similar birth weight may experience various trajectories of foetal growth, where some individuals experience insults at critical periods of foetal development (e.g. organogenesis) while others that experience similar insults at a less critical period. This is supported by Gardner *et al.* (2004)'s study in sheep, where nutritional deficiency at peri-embryo-implantation was associated with reduced brain weight and possibly compromised cardiovascular control for the progeny at one year of age (Gardner *et al.*, 2004). The progeny in both study groups had similar birth weights (Gardner *et al.*, 2004). Therefore low calf sensitivity in teat-seeking in tropically-adapted calves that occurs independent of birth weight (Kim *et al.*, 1988) may be explained by insult to the early conceptus with effects that may ultimately lead to reduced neural or cardiovascular development (Gardner *et al.*, 2004) by neonatal life. Martin *et al.* (2007) subjected *Bos taurus* beef heifers to either given protein supplementation or not during late pregnancy and subsequently were split into groups to be subjected to either higher or lower quality during early lactation. In this study, birth weights did not differ in any of the four treatment groups, though progeny born to dams that were supplemented were heavier at weaning, pre-breeding and their first pregnancy diagnosis (Martin *et al.*, 2007). The difference in progeny performance may be explained by programming of individuals during the foetal stage (Martin *et al.*, 2007). Although supplementation was associated with a higher body condition score at around calving (Martin *et al.*, 2007) and therefore increased progeny growth may at least partially be explained by increased milk yields in early lactation (McBryde *et al.*, 2013) with possible neonatal epigenetic programming (Moura and Passos, 2005, Bagnell *et al.*, 2009). In northern Australian beef breeding herds, the effects of maternal nutrition during pregnancy (including epigenetic effect) on calf vigour are poorly understood.

Yates *et al.* (2012) reported in their review that heat stress on the cow during gestation may lead to placental insufficiency, restricted foetal nourishment and consequently the birth of low-birth-weight, low-vigour calves. A study in Brahmans showed that the relative frequency of low birth vigour varied from 3.5% to 11.8% over a number of years, and that birth-vigour was affected by sequence of birth in the calving season, temperature at birth and the occurrence of precipitation at birth (Wythe, 1970). This study was conducted in Texas, USA, where the hottest calving month was in July with an average temperature of 28^{o}C and the coolest calving month was in January, with an average temperature of 11^{o}C. Month of birth effects may involve both environmental and nutritional components, though nutritional variables were not reported in Wythe's study. Time in the calving season may at least partly explain vigour in standing and suckling (Edwards, 1982), which is also an apparent function of nutritional and environmental risk factors.

In *Bos taurus* cows, heat stress reduced uterine, foetal and utero-placental secretion rates primarily through differences in blood flow (Reynolds *et al.*, 1985). In a *Bos taurus* cows, increased temperature and relative humidity (i.e., (37±1^{o}C and 27±2% versus 21±1^{o}C and 35±10%) within the approximate period of 7 to 15 days after conception was associated with a reduction in conceptus weight, as measured at approximately 16 days after conception (72.8 vs. 157.9 mg), respectively (Biggers *et al.*, 1986). The above studies indicate that heat stress may have implications for foetal development and therefore neonatal vitality. In Holstein cows, shade deprivation during the third trimester of pregnancy was associated with reduced birth weight of calves (Collier *et al.*, 1982). For pregnant ewes exposed to 0, 55 or 80 days of heat stress starting at day 35 of gestation, foetal weights at day ~130 of gestation were 3.4±0.33, 1.84±.03 and 0.88±0.14 kg, respectively (Galan *et al.*, 1999). This study demonstrates how heat stress can effect foetal growth in a cumulative manner (Galan *et al.*, 1999). Despite the possibility that compensatory gain in foetal weight can be made through pregnancy, heat stress at critical stages of development could have 'all or none' type of effect (Funston *et al.*, 2010, Hubbert, 1973). For example, uterine insufficiency during critical stages of neuronal differentiation that may at least partially explain the birth of calves as reported by Kim et al., (1988), where they are born within the normal range for birth weight, yet lack an ability to seek teats and suckle.

The importance of hormonal control over foetal development should not be discounted. Maternal protein intake is associated with maternal plasma IGF-I, IGF-II and IG-FBP (Sullivan *et al.*, 2009b), hormones that may be mediating nutritional effects on foetal growth. Maternal plasma IGF-I at day 271 of gestation was negatively associated with calf crown length at birth (Sullivan *et al.*, 2009b). In Holstein heifers, compared to controls, those that had melatonin included in their diet between days 190 and 262 of gestation had an increase in uterine blood flow of 25%, from day

210 of gestation (Brockus *et al.*, 2016). The role of hormones should be considered for measurement in future calf mortality research.

Results of Kim *et al.*, (1988) demonstrate a trend ($P < 0.1$) where Brahman cows had greater calf grooming scores when the calf was of higher vigour score. Therefore the vigour of calves at birth may be an important driver to prompt dams for mothering behaviour, including allowing the calf to nurse.

2.3.4 Maternal behaviour and milk delivery

Brown *et al.* (2003) and Bunter *et al.* (2013) have reported mismothering and abandoning of calves as a risk factor for calf mortality. In Brown *et al.* (2003)'s extensively-managed herd of Brahman heifers, 4.1% of calves born died due to mismothering and 3.6% of calves born were temporarily abandoned by their dams. These dams were observed frantically looking for their calves for more than a day before finally finding them and raising them normally. Irregular mothering behaviour has been reported to be associated with prolonged time from birth to first suckling, especially in heifers (Rowan, 1992). Therefore, risk factors for calf vigour (as discussed in the previous section) may mediate some of the impact on dam maternal behaviour. Kim *et al.* (1988) reported that a possible reason for cows abandoning their calves is that the dam can lose interest in a calf with the inability to suckle. Improved understanding of risk factors to poor maternal behaviour are required to improve it (Fordyce *et al.*, 2014).

Foetal and calf mortality of 9% was associated with mustering efficiency <90%, i.e. animals yarded as a proportion of number expected (McGowan *et al.*, 2014). This may be explained by the risk of cow-calf separation due to environmental or mustering circumstances. For first-lactation and mature cows, increases in foetal and calf mortality of 9 and 2.5%, respectively, were associated with mustering around months of calving (McGowan *et al.*, 2014). Therefore a disturbance in the maternal bonding process may occur, or alternatively pre-mature calving may be initiated, where those cows experiencing their first calving are most susceptible.

A nervous temperament in cows may be related to increased risk of calf abandonment and therefore dehydration. In Brahman cows, maternal protectiveness score (5 point scale), as measured within 24 hours of birth had no relationship with neonatal mortality (Muller, 2007). Therefore, other detailed measures may be needed to capture the important risk factors for cow maternal behaviour that ultimately impact on neonatal colostrum/milk uptake and survival. Compared to older ewes, first parity ewes would more often abandon, avoid and provide little assistance with their lamb's first effort to suckle (Cloete and Scholtz, 1998). It may be important to run heifers with older cows in order for facilitate their learning as mothers, despite recommendations to separate heifers from cows to feed supplements designed for specific classes of animal (Burns, 2015). An

extreme level of maternal protectiveness, to the point of being nervous, may lead to higher relative frequency of calf mortality (Holroyd, 1987). In dairy cows that were allowed five minutes of bonding time with their newborn calf followed by cow-calf separation, dams would recognise their own calf and start vigorous licking if re-introduced after a period of up to five hours (Hudson and Mullord, 1977). In the same study, a separation period of 24 hours was associated with dams being distressed and lacking the ability to recognise their calf (Hudson and Mullord, 1977). For northern Australian beef breeding herds, few detailed studies have investigated the possibility of calf abandonment where cows may leave their calves to graze or water, which may be exacerbated in situations where stocking rates are low. In some Brahman cows, water consumption increases from 23 to 61 litres as ambient temperature rises from 30^{o}C to 37.8^{o}C (Brody, 1956). Therefore, high temperatures may lead to cows leaving their calves for longer duration or more frequently to travel to water. In a herd of Brahman cows, instinct to nurse the calf was found to be affected by year of calf birth, temperature at birth and precipitation at birth (Wythe, 1970). In summer, cows took longer to lick their calf, compared to winter (Barrier *et al.*, 2012). Therefore, the effects of heat stress on maternal behaviour may be complicated, where increased heat stress may lead to reduced mothering behaviour while the calf is present and may also lead to higher risk of calf abandonment. Both risks may ultimately lead to calf milk deprivation and dehydration. In Brown *et al.* (2003)'s herd of first-lactation Brahman cows, 2.5% of calves born were observed to be suckling dams other than their own, for several days before dying due to dehydration under hot ambient conditions. In the same study, 1% of calves died due to being abandoned at birth.

2.4 Fluid dynamics of neonatal calves

This section addresses current literature on dehydration and rehydration in neonatal calves, and related body water and electrolyte dynamics. The accuracy and practicality of various methods to quantify body water dynamics are discussed.

Hyperhydration is defined as a steady state excess of total body water and hypohydration is a steady state deficit in total body water. Dehydration and hydration refer to the respective processes of losing or gaining body water. Euhydration refers to a state of body water balance. Terrestrial animals are continuously confronted with water loss (Ross and Christie, 1969) through the skin, respiratory tract and intermittent loss through urine and faeces (Greenleaf, 1992). Therefore, euhydration occurs in a dynamic way where the body continuously dehydrates and where fluid is replenished at varying volumes and intervals of time (Greenleaf, 1992). Body water may be lost at a varying rate depending on level of dehydration risk factors present. Fluid losses during dehydration may be classed as insensible (not able to be sensed) or sensible. Insensible fluid losses occur through cutaneous and respiratory evaporation. Sensible fluid losses occur through

urination and sweating. In calves of 97 kg to 201 kg live-weight, average rate of insensible water loss was 5.5% of live weight/day. In the same study, sensible losses by urine output was estimated at 1 mL/kg live weight and losses by faeces were estimated to be 0.51 kg/100 kg live weight/day (McBryde *et al.*, 2013).

Healthy, milk-deprived tropically-adapted neonatal calves develop hypernatraemic dehydration (Fordyce *et al.*, 2015), as do milk-deprived neonatal infants (Çağlar *et al.*, 2005, Sofer, 1993, Poskitt, 2004). In infants, hypernatraemic dehydration almost exclusively occurs in those who are breast-fed and cases are not frequently observed in those who are bottle-fed (Moritz, 2013). Hypernatraemic dehydration describes the change in homeostasis when the fluid lost from the body is lower in solute (principally sodium) content than that of extracellular body fluid. This leads to an increase in tonicity of the extracellular fluid and an osmotically-driven fluid shift from the intracellular compartment to the extracellular compartment (Modric, 2015). This is in contrast to calves that contract gastrointestinal tract infection, diarrhoea and therefore excessive loss of intestinal fluid and electrolytes. Diarrhoeic calves usually experience hyponatraemia (Groutides and Michell, 1990), though there are instances of diarrhoeic hypernatraemia (Abutarbush and Petrie, 2007). Diarrhoea may also lead to a disturbance in the calf acid-base balance. Therefore body fluid dynamics differ between the two types of dehydration, and hydration measures useful for quantifying diarrhoeic dehydration are not directly applicable to quantify dehydration secondary to milk deprivation (Fordyce *et al.*, 2015).

The interventions to prevent and correct dehydration secondary to diarrhoea are well established (Vogels, 2010, Moran, 2008, Anon., 2005, Acres, 1985, Lorenz *et al.*, 2011). The primary cause of calf diarrhoea is exposure to a contaminated environment (Cho and Yoon, 2014). Calves are at highest risk of infectious diarrhoea in situations where there is low area of calving per cow (Radostits and Acres, 1980), poor drainage (Radostits and Acres, 1980, Schumann *et al.*, 1990) and cool moist conditions where all major enteric pathogens can survive for weeks to months (Anon., 2005). In addition, low ambient temperatures and rain may compromise immune response of calves (Cho and Yoon, 2014) and lead to a delayed first standing and suckling of colostrum (Diesch *et al.*, 2011). These risk factors are typical of intensive temperate cattle production systems, especially in dairy cattle. As tropical and subtropical beef production is generally characterised by extensive management and a spring-summer calving, there is likely a low risk of calf diarrhoea. Although there may be surface water present around calving in some situations, this would provide little risk for infection of calves given that animals would generally not be confined to these areas. Given the risk of low calf colostrum and milk uptake in northern Australian extensive beef production systems (described in section 2.3) and low the level of risk to diarrhoea, if dehydration mediated mortality is occurring in these systems, it is likely to be occurring by fluid

deprivation. For the purposes of this thesis, unless otherwise stated, the term *dehydration* refers specifically to hypernatraemic dehydration.

2.4.1 Dehydration and risk of neonatal mortality

In eight healthy Brahman-crossbred neonatal calves under tropical conditions, milk deprivation for one to three days resulted in dehydration exceeding 20% (Fordyce *et al.*, 2015). Some of Fordyce *et al.* (2015)'s calves were not vigorous enough to stand at this level of dehydration. Due to the extensive nature of northern Australian properties, it is generally not practical to locate calves and assist them in standing and suckling. Therefore tropically-adapted neonatal calves in extensively-managed herds may be at high risk of dehydration mediated mortality if they do not ingest milk for a period as short as 2-3 days of (Fordyce *et al.*, 2015). For breeding herds across northern Australia, the frequency at which neonates experience>20% dehydration, or any level of dehydration is not clear.

Additional study is required to confirm if Fordyce *et al.* (2015)'s results apply to other environments and genotypes in northern Australia. In addition, further study is required to quantify the extent of dehydration that occurs with various milk consumption patterns through neonatal life. There are many studies that have investigated the effectiveness of milk or milk replacer in alleviating dehydration secondary to diarrhoea, e.g. Brooks *et al.* (1966) and (Constable *et al.*, 2001). As diarrhoeic dehydration has different effects on calf vigour and fluid dynamics than for calves suffering dehydration secondary to milk deprivation (Fordyce *et al.*, 2015), the fluid dynamics of the latter type of dehydration requires further investigation. The specific thresholds of fluid shift and fluid loss at which milk deprived calves are no longer able to stand and suckle are not fully understood. With clarification on fluid dynamics associated with calf dehydration, interventions to critical points within this biological system may be targeted more precisely. Therefore intervention inputs aimed at increasing calf survival rates may be applied more efficiently and profitably.

2.4.2 Fluid replenishment in neonatal calves

Literature is very limited for calves with hypernatraemic dehydration secondary to fluid deprivation. In humans that were dehydrated to change in plasma volume by -7.5%, with oral rehydration, a 10% increase in plasma volume was detected at 15 minutes post-rehydration (Godek, 2000). The 15 minute measurement was the first measurement for detection of rehydration and there was no further increase in plasma volume at 30 or 45 minutes post-rehydration (Godek, 2000). Therefore, in dehydrated individuals, it is likely that rehydration is a rapid process that occurs before 15 minutes. A similar rate of rehydration may occur in dehydrated neonatal calves, given that they are pre-ruminants and use similar digestive processes to monogastrics (Radostits and Bell,

1970), with similar regulation of abomasal emptying (Sen *et al.*, 2006) into the intestines where the majority of milk water is absorbed (Smith, 1961).

Thirty minutes after fasted calves were fed milk, there was a rapid increase in abomasal outflow that was maintained for the period of 1-4 hours after feeding (Smith, 1962). After 4 hours, there was a decrease in outflow (Smith, 1962). This is consistent with the report of Mylrea (1966), who studied calves of 2-28 days of age and fed them milk at 50 mL/kg live-weight every 12 hours. In this study, digesta flow through the pylorus peaked within an hour of feeding, and was followed by an apparent exponential decrease (Mylrea, 1966). Within a few minutes of feeding, clear watery fluid with a green tinge flowed for 3-4 hours, and was followed by an increasing flow of thick white material from the milk clot (Mylrea, 1966). This is consistent with studies on milk clot formation, where the milk clot (also known as curd) forms in the calf abomasum within minutes of milk ingestion (Moran, 2012). The curd consists mainly of casein protein and milk fat (Logenbach and Heinrichs, 1998) and therefore the flow rate of milk-clot crude protein and fat flows to the duodenum at a slower rate than whey milk components (Petit, 1987). Given that water is not an important component of the milk clot, it may exit the abomasum more freely to be subsequently absorbed in the intestines.

In the calves of Mylrea (1966), digesta as observed at mid-length along the intestines was most often clear in colour, and watery to mucoid in consistency, but also ranged from yellow to golden brown in colour. At mid-length along the jejunum, flow rate peaked within the 4-5 hours after feeding, indicating the delay in flow from the pylorus (Mylrea, 1966). Ileal contents were dark brown and moderate to thick in consistency (Mylrea, 1966). Based on the findings of Mylrea (1966), most of the absorption of digesta occurs in the distal half of the small intestine, at an approximate delay of 4-5 hours after feeding. This harmonises with the findings of Smith (1962), demonstrating that 80 – 95% of milk water, chloride and potassium is absorbed in the small intestine, followed by further absorption of these substances in the large intestine. In a study on humans, digesta was flushed from the colon, and thereafter an introduced urea and electrolyte solution was introduced where only 5.2±0.6% of introduced urea was only absorbed per hour (Wolpert *et al.*, 1971). Water absorption paralleled urea absorption (Wolpert *et al.*, 1971).

Additionally, in calves, the majority of water of ingested milk rapidly passes into the intestines after feeding (Mylrea., 1966), while much milk protein and fat clots in the abomasum (Logenbach and Heinrichs, 1998) to slowly pass into the intestines (Petit, 1987). In addition, urea, which is soluble in water when injected into the bloodstream, will equilibrate through tissues within an hour (Dalton, 1964). The above are suggestive of rapid (likely instantaneous) absorption of water from ingested milk, especially in dehydrated calves. The above studies are also suggestive that in dehydrated calves, efficiency of converting ingested milk water to body water is 100%, until

full hydration is reached. The total water available for the calf may to be equal to the volume of milk consumed, given that milk solids have been reported to produce an amount of metabolic water similar to the weight of milk solids (Macfarlane *et al.*, 1969).

In humans, exercise does not lead to appreciable change in rate of gastric emptying (Fordtran and Saltin, 1967). In another study on exercising humans, gastric emptying rate was lower in hot conditions (49^{o}C) than in warm conditions (35^{o}C) (Neufer *et al.*, 1989). Given these reports, it may follow that thermal strain associated with exercise and heat stress may also impede gastric emptying rate in neonatal calves.

2.4.3 Direct impact of temperature and humidity on hydration

Cattle may dissipate heat load by evaporative or non-evaporative means (Finch, 1985). For cattle experiencing heat stress, increasing the rate of cutaneous evaporation is the main (Finch, 1985) and most effective (Brody, 1956) mechanism of heat loss. Cattle can lose 2.43 joules or 0.58 calories per kilogram of water evaporated (latent heat of vaporisation; Brody, 1956). In tropically-adapted neonatal calves, higher heat load increased rate of dehydration, with a rate of body water loss of 1.1% per hour at 39^{o}C (Fordyce *et al.*, 2015). Other studies describing impacts of ambient temperature on calf dehydration are limited. Although, the results of Fordyce *et al.* (2015) are consistent with Brahman cows under controlled conditions, for which an increase in ambient temperature from 10^{o}C to 42.3^{o}C, was associated with an approximate 2.8 fold increase in evaporative heat loss (Brody, 1956). In these cows, thermoregulatory mechanisms became active at approximately 27^{o}C and started to fail at approximately 35^{o}C (Brody, 1956). This is further supported by a study on 15-month-old Brahman steers that were subjected to 10 days of either thermo-neutral or heat stress conditions (22^{o}C vs 41^{o}C), with respective evaporative losses of 1.7 and 6.4 litres per 100 kg live weight (i.e., a 3.8 fold increase; O'Kelly and Reich, 1981). This is further supported by a study on Brahman and Brahman crossbred steers, where ratio of metabolic heat loss by evaporative means:non-evaporative means was 75:25% when ambient temperature was high enough that it approached skin temperature (Finch, 1985). Increases in this ratio were associated with increases in ambient temperature, especially at ambient temperatures exceeding skin temperature (Finch, 1985). Skin temperature of Brahmans steers in a tropical environment was reported to be approximately 34^{o}C (Moran, 1973). When ambient temperature exceeded skin temperature by 7^{o}C, evaporative heat loss as percentages of non-evaporative heat loss were ~190% and ~150% for Brahman crossbred and Brahman steers (Finch, 1985). Therefore cattle of higher *Bos indicus* content are more efficient at heat dissipation by evaporation.

Hydrated Zebus began to sweat and pant simultaneously at approximately 31^{o}C, though in dehydrating Zebus evaporative losses did not occur until a higher threshold temperature of 35–36^{o}C

(Finch, 1973b). Changes in skin temperature on the back of the animal were associated with changes in both cutaneous and respiratory rates of evaporation. Rectal temperature was not associated with rate cutaneous or respiratory evaporation (Finch, 1973b). In hydrated Zebus, evaporative losses had much greater associations with skin temperature than rectal temperature. Skin temperature and rectal temperature accounted for 80 and 25% of variation in evaporative losses, respectively. Similar associations were found in dehydrated Zebu, though rectal temperature tended to have a stronger association with evaporative water loss (Finch, 1973b).

In a study of Brahman steers, rate of cutaneous evaporation increased with ambient temperature, but not humidity (Finch, 1985). Respiratory water loss also increased with temperature, though humidity had a negative effect. Finch (1985) reported that in cattle, high respiratory rates are related to severe heat stress that cannot be alleviated at sufficient rate by other heat loss mechanisms. When cattle are subjected to high heat loads, 15% of the heat lost from the body occurs by removal of heat from the body core by respiration (Finch, 1986). Failure of thermoregulation is known to have a negative impact on production in adult cattle, given that it's associated with rapid increase in rectal temperature, decline in feed consumption and live weight (Brody, 1956). In animals standing in the sun throughout the day, peak rates of evaporative water loss coincided with times of maximum solar radiation (Finch, 1973b). Calves may also experience these changes in thermoregulation physiology at similar critical temperatures or heat loads.

In hot conditions, cattle may divert increased amounts of blood towards cutaneous vasculature to support increased heat and water loss via sweating (Johnson and Hales, 1983). At ambient temperatures exceeding 40^{o}C in cattle, apocrine sweating may be important form of cutaneous evaporation, with increasing rates of secretion at temperatures above 40^{o}C (Ferguson and Dowling, 1955). The sodium and potassium content of cattle sweat were reported to be less than 1 – 3% of intake of these electrolytes in feed (Johnson, 1970). Therefore, loss of electrolytes through sweat is plausibly of little significance to alterations in body fluid dynamics during dehydration of a calf. This is consistent with the report of hypernatraemic dehydration in milk-deprived neonatal calves (Fordyce *et al*., 2015).

2.5 Observing and quantifying hydration status in neonatal calves

It is very difficult to accurately quantify fluid balance, even with neonatal infants in hospital conditions (Asperen *et al.*, 2012). In addition, defining the threshold between euhydration and hypohydration is not simple as it must be based on euhydration (Shirreffs, 2003), which is a dynamic state of water balance. For neonatal calves, a useful baseline for euhydration may be 74±3% body water (as a percentage of live-weight), as it was quantified in fully hydration healthy calves (Fordyce *et al.*, 2015) and is consistent with other reports including Haigh *et al.* (1920)

andDalton (1964). Therefore one possible definition of hypohydration is that in calves that are significantly lower than the above value. A more useful definition may be the point of hypohydration at which calves are at high risk of an inability able to stand and suckle. This point is poorly defined, although the study of Fordyce *et al.* (2015) reported that in 8 Brahman-cross calves, some of those that had reached 20% dehydration were no longer able to stand and suckle. Further research is required to confirm this point of hypohydration in other genotypes and environments. This point would likely provide enough differentiation between calves, in research designed to further understand how risk factors are impacting on calf dehydration under field conditions. The below sections describe the accuracy, practicality and therefore usefulness of various laboratory, subjective and objective non-invasive measures of hydration.

Change in body weight is a highly-useful measure for quantifying fluctuations in hydration status in humans, and has been used in research as the most accurate measure when developing indicator measures of hydration (Armstrong *et al.*, 1998, Asperen *et al.*, 2012, Hartnoll, 2003). Acute change in body weight over a short period may be assumed to be explained by change in body water in many circumstances, given that other body components would not change at a similar high rate (Shirreffs, 2003). In non-suckling neonatal calves, live weight was shown to decrease by about 7% daily under conditions where ambient temperature was not extremely high (Fordyce *et al.*, 2015). This is equivalent to approximately 2.5 litres of milk per day (Fordyce *et al.*, 2015). Based on the above studies, it may be assumed that in suckling calves dehydrating due to milk deprivation, weight loss over a short period can be attributed to fluid loss, or weight gain over a short period may be attributed to milk uptake, although over longer periods, tissue loss or gain must be accounted for. Therefore, though the weigh-suckle-weigh technique may be useful as a single indicator of hydration, even measures over 12-24 hours will include a component of tissue accretion. The efficiency of tissue accrual or catabolism in tropically-adapted calves is not clear. In a study on infants where increase in weight was tested as a measure of milk intake (with known intake volumes), estimates were imprecise (overestimations and underestimations of up to 30 ml), emphasising the importance of using scales with sensitivity greater than what is required for simple single weighing of individuals (Savenije and Brand, 2006). McBryde *et al*. (2013) compared using the weigh-suckle-weigh technique with separation of cows and calves for periods prior to suckling of 24 hours and were weighed regularly over 24 hours and "calf weights were adjusted for urination, defecation and calculated insensible water loss". McBryde *et al*. (2013) showed that the measurement using the weigh-suckle-weigh after a period of non-suckling of 24 hours does not provide sufficient accuracy to account for fluid losses through urine, faeces and insensible means.

In humans, tracer dilution is the most common method of quantifying total body water (Shirreffs, 2003), with compounds including tritiated water or urea. Compared with tritiated water,

urea solutions are easier to prepare and are analysed with greater ease (Geerken *et al.*, 1988a). Therefore urea is a more practical alternative to tritiated water for field research. For field research, where animals are measured far from laboratory facilities, the urea space method is highly suitable given that it considered precise enough to quantify body water and can be used in the field, where potential risk factors to dehydration exist, in a cost effective way (Fordyce *et al.*, 2015). There is some error associated with using urea in the dilution method, which may be explained by urea being a major body metabolite. For example, in two-month-old nursing foals, mean urea space was 4% less than tritiated water space. Standard deviation for the proportion of water to body weight was higher with the urea space (3.8%) than tritiated water space (1.6%) (Geerken *et al.*, 1988a).

In series testing of univariate predictors for hydration status, in hypernatraemically dehydrating calves, the predictor explaining the most variance was sodium (R^2=0.69%; Fordyce et al., 2015). Serial measures of serum sodium are useful to monitor changes in hydration status during the first few days of life in infants (Hartnoll, 2003). In a New Zealand dairy herd, milk sodium concentration during early lactation (as measured when) did not differ, with concentrations in spring, summer, autumn and winter consistently at 0.45 (Auldist *et al.*, 1998). Therefore, the combined results of Fordyce et al (2015) and Auldist et al (1998) suggest that plasma sodium is a useful indicator of milk uptake and hydration in young calves. A possible source of error in using plasma sodium concentration as a predictor of hydration may occur if renal regulation aims to conserve sodium at greater degrees of dehydration (Francesconi *et al.*, 1987). If this error exists, a means to reduce the error may be to quantify the sodium concentration of both plasma and urine.

In serial testing of univariate predictors for hydration status, the predictor achieving the second highest adjusted R^2 was urea at 0.62% (Fordyce *et al.*, 2015). In infants, urea also reflects nutritional status and nitrogen load (Hartnoll, 2003). This may also apply to measuring hydration status in neonatal calves. The mixed model of (Aguilar *et al.*, 2012) demonstrates how milk urea nitrogen (mg/dL) in multiparous Holstein and Holstein-cross-Jersey cows is partly explained by cow milk yield (kg/d) and cow crude protein intake (kg/d), with respective parameter estimates of -0.33 and 20.3. Therefore, in neonatal calves, the negative association between plasma urea concentration and hydration may be partly confounded, if there is a positive association between plasma urea concentration and milk uptake. Despite this, in reality, neonatal calves of low milk uptake in northern Australia are likely receiving barely any milk (Fordyce *et al.*, 2015), which would negate any possible confounding effect of milk uptake on plasma urea concentration.

In colostrum, a major component of total protein is maternal IgG antibody or globulin (6-12% of total protein), though within 48 hours post-partum, the globulin content of milk secretions reduce to a similar or lower quantity than albumin, with a corresponding reduction in total protein content (Crowther and Raistrick, 1916). Plasma proteins do not leave the vasculature

readily(Armstrong *et al.*, 1998), and therefore neonatal calves that ingest colostrum may have a corresponding level of plasma globulin and total protein, which is maintained beyond colostrum production of the cow. This is consistent with a study on neonatal calves that demonstrated a marked increase in serum protein concentration within two hours of feeding (Danijela *et al.*, 2002). In the same study, feeding of 3 or 1.5 litres of colostrum during the first 12 hours of life led to differences in serum protein concentration at 4 hours post-feeding (54.7 g/L and 42.7 g/L, respectively) and at day 7 of life (69.5 g/L and 60 g/L, respectively; Danijela *et al.*, 2002). A limitation in the use of plasma protein or globulin concentration as an indicator of volume of colostrum uptake may be that there is decreasing efficiency of IgG absorption by calves, where every 30 minute delay of first colostrum decreases calf serum IgG concentration by about 2 mg/mL (Rajala and Castren, 1995). In addition, colostrum IgG concentration is influenced by colostrum yield of cow, cow parity and season (Conneely *et al.*, 2013). Despite the value of globulin as an indicator of colostrum ingestion in hypernatraemically-dehydrating neonatal calves, plasma protein concentration only predicted hydration status with an adjusted R^2 of 0.18 (Fordyce *et al.*, 2015). This result of Fordyce *et al.* (2015) should not discount the value of globulin as a measure of colostrum uptake, as these calves had likely received very little colostrum. Plasma globulin is likely a useful measure for the detection of rehydration that occurs by colostrum uptake.

The ingestion of colostrum in young calves was detected with the presence of protein in the urine, especially within the period of 10-40 hours after birth (McDougall, 1964). This is consistent with a study on infants that demonstrated decreasing urine protein concentration during the first three days of life (Svendsen and Tveterås, 2008).

Physical calf measures have been tested as predictors of hydration, and these estimates are imprecise. Examples of predictors for hydration status include: oral mucous moistness (adj. R^2= 0.31), eyelid tenting time (adj. R^2= 0.44), ability to stand score (adj. R^2= 0.4), alertness score (adj. R^2= 0.47) and kg live-weight/mm hind-leg length (adj. R2= 0.4; Fordyce *et al.*, 2015). Therefore these measures may not allow precise quantification of hydration though may be suited to large field research projects with sufficient number of calves to provide required statistical power.

2.5.2 Development of the urea space technique in calves

In a study on fasting dogs with *ad libitum* access to water, a urea solution injected intravenously appeared to distribute equally throughout the total body water at approximately one hour after injection (Painter, 1940). In this study, from the point of intravenous injection of a urea solution, there was a rapid rate of decrease in blood urea concentration, associated with the diffusion of urea out of the blood vessels into other body water compartments. At approximately one hour after urea injection there was a reduced and approximately constant rate of decrease in

blood urea concentration. At one hour after injection, it was presumed that the injected urea had distributed evenly throughout the total body water. It was also presumed that the rate of decrease in plasma urea from one hour after injection reflected the rate of urea excretion from the body. Based on this logic, the rate of urea excretion from one hour after injection was extrapolated back to the time of injection, to give the concentration theoretically achieved with instantaneous even distribution of injected urea throughout the total body water before any urea excretion occurred (C_0). This relies on the assumption that the rates of excretion are the same during the distribution of urea and the following excretion only phase (Painter, 1940).

The methods of Painter (1940) were employed in calves, where apparent time for urea to equilibrate throughout the total body water was also one hour. The weight of injected urea can be divided by C_0 to calculate volume that the urea has access to within the body fluid (Painter, 1940, Dalton, 1964). This volume is referred to as urea space (Painter, 1940, Dalton, 1964).

It was reported that in 12 adult cattle, urea distributed through the body within 9–10 minutes of injection to give a plasma urea value which is nearly the same as that obtained from extrapolating plasma urea disappearance curves back to the time of injection (Preston and Kock, 1973). This study was not focussed on urea space methodology to measure body water but rather body composition. In Dalton (1964)'s study, it was found that for the majority of calves, appeared to equilibrate through the urea space after an hour. For some instances in the same study, the blood urea concentration at one hour after injection was slightly above the fitted line of representing the exponential rate of urea disappearance from the calf body (Dalton, 1964).

The 12-minute sampling urea space method has been used to quantify body composition in adult cattle (Hammond and Rumsey, 1990) and hydration status in young, milk deprived calves (Fordyce et al., 2015). The 12-minute urea space method is suitable for field research given that it is less laborious than other urea space methods that require the collection of multiple blood samples at specific time intervals post-urea infusion. The 12-minute urea space technique on apparently fully-hydrated calves predicted body water values that are consistent with body water values quantified using other methods (Fordyce *et al.*, 2015).

In the urea dilution technique, fluctuations in the endogenous urea level were thought to interfere with the accurate measurement of the disappearance curve of exogenous urea (Painter, 1940). In five healthy calves, it was concluded that hourly plasma urea concentration fluctuations did not fluctuate to an extent that it was considered a significant source of error for the urea space method (Dalton, 1964). There are some studies that have reported abnormally high or low urea space values, with no specific explanation. There is clearly variation inherent in the urea space technique. The slight deviation of some calves from the generally-known exponential decrease in infused urea is likely not a major source of error in the 12-minute urea space technique. Urea space

had a low precision in this study (SE of prediction of 15.7%) and therefore regression equations were required to calculate full hydration values (Fordyce *et al.*, 2015).

2.5.2.1 Urea molecule distribution

Calculated urea space values were not different between fed calves and calves starved for 18 hours (Dalton, 1964). This is evidence that there is no significant distribution of infused urea into milk water held in the abomasum. In the same study, starved calves had overestimated urea space as a percentage of live weight compared to fed calves. This was due to the fact that starvation in the calves led to no change in urea space values though led to a decrease in live weight (Dalton, 1964) (i.e., starvation decreased the denominator of live weight though did not affect the numerator of urea space). Fasting in calves increased calculated urea space as a percentage of live weight by approximately 2.5% (Dalton, 1964).

Urea will only enter the intestinal lumen from the bloodstream at low rates. In sheep and goats, urea was reportedly endogenously secreted into from the bloodstream into the colon at a rate of 0.10 – 0.31 mmol/hour and this rate was proportional to how much urea was injected into bloodstream (Engelhardt *et al.*, 1984). In rats, urea was secreted into the proximal and distal colon at respective rates of approximately ~ 8.5 µL and ~5.5 µL (per minute-gram wet weight of intestinal tissue; Fihn and Jodal, 2001). Urea absorption into the colon might be minimal at best (Billich and Levitan, 1969) compared to that of the small intestine (Fordtran *et al.*, 1965).

The above studies indicate that the urea space is not equivalent to total body water, as a urea solution injected into the bloodstream has poor access to gut compartments including the abomasum and large intestine, with apparently minimal access for diffusion into the small intestine.

2.6 Literature review conclusion

A collective of studies indicate that many calf mortalities in northern Australia may be explained by milk deprivation and dehydration within the first week of life. Research across northern Australia has demonstrated large losses between pregnancy and weaning that are consistently associated with nutritional, management and environmental risk factors. Detailed studies indicate that the impacts of the risk factors are occurring through low milk delivery to calves, especially during neonatal life, and that milk delivery is driven by timely milk production and calf ability to suckle.

Although the general risk factors associated with calf mortality are likely to be specifically explained by calf dehydration, no study has investigated risk or the frequency at which dehydration mediated calf mortality may occur. Several methods to quantify calf hydration are available.

2.7 Thesis hypotheses

The main hypothesis is that calves vary in their hydration status as measured on a particular day of neonatal life, which would indicate variation between calves in their trajectory of hydration status throughout neonatal life. A second hypothesis is that specific nutritional risk factors are driving neonatal calf hydration status, along with animal factors including maternal protectiveness and teat conformation. A third hypothesis is that neonatal calves exposed to risk factors for dehydration dehydrate at a rate that leads to a fatal level of body water loss during neonatal life. The above hypotheses have not been studied in extensively managed breeding herds of northern Australia. Variation in calf hydration would indicate opportunity to develop interventions aimed at decreasing the relative frequency of calf dehydration and therefore calf mortality.

2.8 Thesis objectives

The overall objective of this thesis was to assess evidence for dehydration as a risk factor to calf mortality in northern Australia. This was achieved in four ways: 1) Literature review on the possible risk factors for calf dehydration and practical methods to quantify dehydration and its risk factors, 2) Detailed measurements on hydration status at each day of neonatal life, 3) Large scale measurement of risk factors to calf mortality that occur during gestation and on day of birth in two low-calf mortality situations, and 4) Assessment of how rapidly calves could experience dehydration mediated mortality, with an interactive theoretical model.

3. Variation in milk delivery by Brahman cows in the days after calving

3.0 Abstract

The aims of this study were to describe how calves vary in their hydration profile through neonatal life and to quantify the relative frequency of neonatal calves that experience dehydration. The study included 12 neonatal Brahman calves at Spyglass research site (dry tropics, northern Queensland). Change in live-weight from birth was used as the primary measure of hydration. Half of this cohort had low live weight gain between birth and day 3 of life ($p = 0.01$) compared to calves growing at 0.95±0.24 kg/day from birth. Low growth and associated reduced plasma protein ($p = 0.04$) in 6 calves indicated reduced colostrum, milk uptake and therefore reduced body fluid replenishment compared to other calves. This positions them at high risk of mortality under adverse environmental conditions. Urea space was tested as a measure of body water; however, percentage body water estimates were unrealistically elevated (> 80% of live weight) and therefore not representative of actual calf percentage body water.

3.1 Introduction

Within cows that achieve pregnancy across northern Australia, median incidence of foetal and calf mortality ranges from 5% (for first lactation cows in the southern forest) to 16% (for heifers in the northern forest; McGowan *et al.*, 2014). Interquartile ranges for incidence of foetal and calf mortality were between 5% and 14% (McGowan *et al.*, 2014). The variation in incidence of foetal and calf mortality indicates that there may be scope to increase foetal and calf survival rate in many breeding herds, with consequent increases in production and profit. Across mammalian species, the most critical stage of life is the neonatal period (within a week of birth), a time at which they are at high risk of inadequate milk consumption (Nowak *et al.*, 2000). Tropically adapted beef breeding herds are no exception, as during the period from positive pregnancy diagnosis to weaning, 67% (Bunter *et al.*, 2013) and 43.8%[2] (Holroyd, 1987) of mortality occurred during neonatal life. Therefore, much of the variation in foetal and calf mortality incidence may be explained by variation in incidence of neonatal mortality. The reasons for the high risk of mortality during neonatal life are poorly understood (Bunter *et al.*, 2013, Holroyd *et al.*, 2005).

In dairy cows, milk yields during early lactation are strongly impacted by dietary energy (Coulon and Remon, 1991) and can be limited with deficiencies of protein (Cowan *et al.*, 1980). In tropically-adapted first-lactation cows, a low phosphorus diet (0.08, compared to 0.22 g P/kg)

[2] Although neonatal calf mortality rates were not reported in Holroyd (1987), counts were reported for number of pregnancies studied (3285), total losses between pregnancy diagnosis and weaning (516), calf mortality within 48 hours of life (170) and calf mortality between hour 48 and day 7 of life (56). Therefore neonatal mortality rate was calculated as percentage of total foetal and calf mortalities that occur during the first week of life.

during late gestation was associated with an average decrease in early lactation milk production of 1.6 L/day (Castells *et al.*, 2014). Similarly, during early lactation a low phosphorus diet (0.08 compared to 0.28 g P/kg) was associated with an average decrease in early lactation milk production of 2.2–2.9 L/day (Castells *et al.*, 2014). Daily lactation yield tended to decrease by an average of 1 kg for each unit decrease in cow body condition score (1–5 scale) (McBryde *et al.*, 2013). Therefore, increases in foetal and calf mortality associated with low cow protein intake, low cow body condition or inadequate phosphorus (McGowan *et al.*, 2014) may be explained by reduced lactation yields of dams, reduced milk delivery to their calves and consequently calf dehydration and mortality.

In Brahman cows, there was an apparently linear decline in milk yield as ambient temperature increased from 35^{o}C to 43^{o}C (Brody, 1956). This increase in temperature appeared to explain a drop in milk production of about a kilogram. In dairy cows, increased heat stress during late gestation was associated with reduced mammary cell proliferation, as measured 20 days prior to calving, and reduced subsequent average daily milk production (Tao *et al.*, 2011). In dairy cows, temperature-humidity has a two day lag effect on daily milk yield, where an increase in black globe temperatures from 25^{o}C to 35^{o}C appeared to be associated with a decrease in daily milk yield of approximately 2.4 L (Collier *et al.*, 1981) and where change in milk yield (kg) per increase in temperature-humidity index unit was -0.88 for Holsteins and -0.6 for Jerseys (West *et al.*, 2003). Temperature-humidity index >79 for >14 days around calving was associated with a four to seven percent increase in foetal and calf mortality, depending on land type (McGowan *et al.*, 2014). Therefore, the impact of heat stress around calving on foetal and calf mortality may also be partially mediated by reduction in milk yields and milk delivery to calves.

In breeding herds within low stocking density situations, calves may be at increased risk of dehydration. Low stocking density is generally characterised by increased distances for cows to travel (e.g. to water), where calves may either suffer increased rate of dehydration while following dams for large distances, or if the dam is absent from calf during travel, there may be extended periods without milk uptake.

In dairy cows, ample milk secretion does not occur until the days pre-partum (Hartmann, 1973) and therefore if beef cows in extensive environments initiate lactation at a similar stage, then milk availably for the newborn calf may be limited where risk factors for reduced milk production are high during this period. In addition, Fordyce *et al.* (2015) reported that in tropically adapted neonatal calves, 1-3 days of milk deprivation can result in dehydration exceeding 20%, at which point some calves did not recover without assistance. Therefore a discrepancy between early lactation yields and neonatal calf fluid requirements may explain many calf mortalities.

This study investigated the variation in hydration status between calves during neonatal life. If a high relative frequency of calf mortalities is associated with dehydration during neonatal life, then the level of susceptibility of cows and calves to the risk factors for lactogenesis, milk yield and suckling will have a major bearing on losses. The relative frequency of dehydrated calves would provide an indicator of how prone calves are to mortality when the risk factors for milk delivery are present. The hypothesis, that there is a high relative frequency of neonatal calves that experience dehydration, has not been studied in extensively managed herds of northern Australia. Variation in calf hydration would also indicate opportunity to develop interventions aimed at decreasing the relative frequency of calf dehydration and therefore calf mortality. The experimental objectives were to quantify the trajectory of hydration that occurs through neonatal life, whether these trajectories differ between calves, and to verify the measures of hydration in Brahman calves, under field conditions.

3.2 Materials and Methods

Ethics approval

This study was granted animal ethics approval by the Department of Agriculture, Fisheries and Forestry ethics committee on the 19/August/2013 (Application number SA 2013/08/441). This study complied with Queensland's Animal Care and Protection Act (2001) and the Australian Code of practice for the Care and Use of Animals for Scientific Purposes (7th edition, 2004).

Animals and environment

This study was conducted at Spyglass Beef Research Facility situated in the dry tropics of northern Queensland (-19.49 S, 145.68 E), early in the typical calving period for beef cattle of the region (30/August/2013 – 16/September/2013). Twenty Brahman calves and their dams were assessed. Cows used for the experiment were those first to calve within the cohort, with no other specific selection criteria to be enrolled in the study. All cow and calf measures were recorded once daily, between approximately 6:30 am and 12:00 am, throughout neonatal life (first seven days from birth) of each calf. In the weeks prior to and during this study, cows grazed on 95 hectares of northern forest country of predominately native kangaroo grass (*Themeda triandra*), black spear grass (*Heteropogon contortus*) and wiregrass (*Aristida sp*.). Cows were allowed *ad libitum* access to a urea-based supplement (NQ Breeder®, Stocklick trading), that was formulated for late-gestation cows within this environment. Approximate composition of supplement was: 30% urea, 30% salt, 2% phosphorus, 4% calcium, 3% sulphur with a minimum of 6.7% crude protein.

Cows were on average 5.1±0.9 years of age at calving. Cow body condition score during the study was 2.8±0.4 (5-point scale, see below for details). The cattle yards used for study activities were adjacent to the study paddock. Water was available in a trough adjacent to the yards.

Paddock measurements

At approximately 06:30 – 09:30 am daily, dam and calf behaviours were observed for several minutes at a sufficient distance not to disturb them. Subjective 5-point scale measures included:

Maternal protectiveness

-1=ignorant of calf, 3=paying attention to calf from distance, 5=extremely protective of calf)

Calf vigour/alertness

-1=very weak, 3=moderate vigour and alertness, 5=highly vigorous, difficult to capture and restrain)

Calf para-lumbar fossa distension

-1=very sunken, 3=half distended, 5=fully distended, para-lumbar fossa cannot be distinguished visually)

Cow body condition score

-1=very poor, 2=backward condition, 3=moderate, 4=forward and 5=obese

Binary characteristics measured (0= characteristic not present, 1= characteristic present) included calf persistence to suckle, calf bunting udder, milk residue on calf muzzle, dam bellowing, dam kicking calf and milk residue on teats. Each cow-calf pair was walked to the yards after their paddock measures were recorded.

Yard measurements: Calves

For each calf, an initial heparinised blood sample of approximately 6 mL was obtained from the jugular vein and placed in an ice-water bath, as were all other blood samples prior to centrifugation.

Hydration status of calves through neonatal life was measured using the urea space technique, where urea is used as a marker to estimate total body water volume. Urea space is the volume in which injected urea distributes through the animal's body water (Hammond and Rumsey, 1990) and has been used as a practical way to estimate calf total body water volume (Dalton, 1964) and therefore body water as a percentage of live weight. Immediately after initial blood collection, calves were weighed and injected with 20% (w/v) urea solution in 0.9% phosphate buffered saline at rate of 130 mg/kg live weight (Hammond and Rumsey, 1990).

The injected urea was allowed to disperse throughout the calf's body water for 12 minutes before a second blood sample was obtained. Time from blood sample collection until centrifugation and plasma collection was approximately 1–4 hours. This varied between days. Plasma samples were frozen. The urea space technique estimates total body water using the increase in plasma urea concentration between pre- and 12 minutes post-urea infusion. Smaller increases in plasma urea concentration reflect a larger space of distribution, i.e., urea space.

In lateral recumbency, calves were measured for front leg length (elbow to hoof base in the extended foreleg), back leg length (major trochanter of the femur to hoof base of the extended hind leg) and girth (immediately behind forelegs).

Temperatures were measured, including oral temperature (between upper lip and jaw), rectal temperature and ambient temperature under shade.

Physical indicative measures of hydration included:

Eyelid tenting time

-soft pinch the upper eyelid, in cranio-posterior alignment and measure number of seconds for eyelid to return to normal shape

Eyeball recession within eye socket

-mm gap at medial canthus

Suckling reflex

-0=no reflex, 1=reflex only just strong enough to be detected, 3=weak suckling reflex that would only extract small quantities of milk from a functional udder over prolonged periods, 5=highly vigorous reflex

Calf mucus membrane tackiness

-1=very dry and tacky, 2=some moisture detectable by feel, 4=moisture easily felt with little tackiness, 5=calf salivating normally

Plasma variables have been demonstrated to be useful measures of hydration (Fordyce *et al.*, 2015). Therefore, pre-injection plasma samples were tested for various variables. Plasma parameter concentrations were determined using an Olympus analyser (AU400, Beckman Coulter Inc., Brea, California USA).

Plasma variables assessed included:

Plasma total protein concentration (g/L)

-determined by photometric absorbance of a violet complex, which forms after added cupric ions react with sample proteins and peptides

-the absorbance of the violet complex at 540/660 nm is directly proportional to the sample protein concentration

Plasma albumin concentration (g/L)

-was determined by adding bromocresol green and measuring the photometric absorbance of the albumin-bromocresol green complex bichromatically (600/800 nm), as this absorbance is directly proportional to the albumin concentration in the sample

Plasma globulin concentration (g/L)

-calculated as total protein minus albumin

Plasma glucose concentration (mmol/L)

-determined by the enzymatic UV test (hexokinase method)

-in the presence of added hexokinase, adenosine triphosphate (ATP) and magnesium ions, glucose is phosphorylated to produce glucose-6-phosphate and adenosine diphosphate (ADP)

-added glucose-6-phosphate dehydrogenase specifically oxidises glucose-6-phosphate to gluconate-6-phosphate with the concurrent reduction of NAD^+ to NADH

-absorbance at 340 nm was measured, as its increase is proportional to sample glucose concentration.

Plasma bicarbonate concentration (mmol/L)

-quantified using the reduction in absorbance of an enzymatic reaction mixture measured bichromatically at 380/410 nm, which is proportional to the sample bicarbonate concentration

-the reaction mixture included phosphenolpyruvate, phosphoenolpyruvate (PEP) carboxylase, malate dehydrogenase (MD), and nicotinamide adenine dinucleotide (NADH)

Plasma lactate concentration (mmol/L)

-quantified photometrically, where the colour intensity of the final reaction is proportional to the sample lactate concentration

-the initial reaction is the oxidisation of L-lactate to pyruvate and hydrogen peroxide by lactate oxidase

-the final product comes from a reaction of hydrogen peroxide, peroxidase, 4-aminoantipyrine and a hydrogen donor

Plasma urea concentration (mmol/L)

-measured by a rate of decrease in NADH absorbance in a final reaction

-the initial reaction is the hydrolysis of urea with urease and water, which produces ammonia and carbon dioxide

-the final reaction is the production of glutamate and NAD^+ from a combination of the ammonia and 2-oxoglutarate and NADH, with the presence of glutamate-dehydrogenase

Plasma Na^+ and K^+concentrations (mmol/L)

-were determined using crown ether membrane electrodes, specific to each ion

Plasma Cl^- concentration (mmol/L)

-determined using a molecular oriented PVC membrane

-the Nernst equation of a specific ion was used to develop the electrical potential

-an internal reference was used to calculate voltage from the electrical potential, and then calculate the sample ion concentration.

Yard measurements: Dams

Subjective scores 5 point scores for udder size were recorded (Figure 1), in addition to more detailed measures on udder quarters and teats.

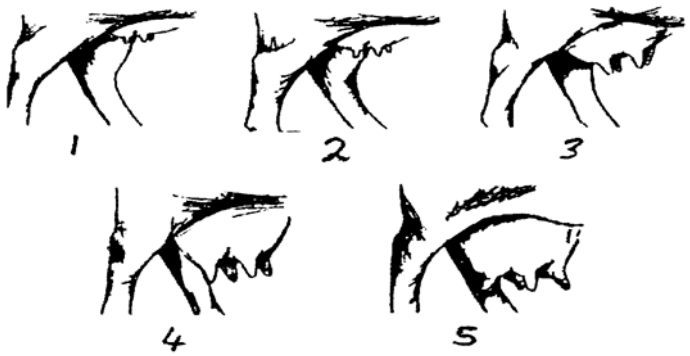

Figure 1. Udder size scoring system (Bunter *et al.*, 2013)

Udder distension (1=completely sucked out and therefore wrinkly in appearance, 2=udder appears to contain a small volume of milk, with little apparent distension, 3=level of distension that results from a calf suckling intermittently as required under benign environmental conditions, 4=udder appears almost full, though not completely tight, 5=completely full, to the point where it appears painful to the cow.

Udder distension was also measured in a non-visual way, by gently palpating the udder quarters with a broomstick. The same scale of distension was used as above.

Udder suspension (1=very loose and pendulous attachment, very weak median suspensory ligament, 2=loose attachment, weak median suspensory ligament, 3=intermediate attachment,

4=tight attachment, pronounced median suspensory ligament, 5=very tight, well defined separation of left and right udder quarters by median suspensory ligament.

Udders that appeared to follow a natural shape were recorded as soft and those that seemed to have inconsistency in shape (e.g. a lump) were recorded as hard. Quarter levelness was recorded, being the vertical distance (cm) between two udder quarter bases most distant from each other.

Teats were objectively measured for width at the midpoint of each teat (mm). Teats were visually classed on shape as cylindrical, conical or bottle shaped.

Data management

It was calculated that a minimum sample size of 20 calves is required to accurately assess the hydration related variables, live-weight and urea space. This minimum sample size was calculated on best available data at the time on tropically-adapted calf dehydration, Fordyce *et al.* (2015). For the calculation, minimum detectable differences within the sample, for a particular day of neonatal calf life, were: 2.2 kg (sd 4.7) for live-weight and 4.8% (coefficient of variation 10%) for urea space as a percentage of live weight. No treatments were included in the study design. For 8 calves, there was missing data for at least 2 days consecutive, precluding interpretation of change in live weight or other measures. These 8 calves were therefore excluded from analyses (except for investigation into the one dehydrating calf).

Analyses

Urea space, as the estimate of total body water volume (L), was calculated as:

Quantity of urea injected (g) / Increase in plasma urea between urea-solution infusion and 12 minutes post infusion (g/L)

In order to capture specific thresholds where risk factors lead to high risk of calf morbidity, a teat difficulty to suckle score (0/1) was calculated for cows of median teat diameter of ≤35 or >35 mm, respectively. In addition, an udder difficulty to suckle score was calculated, where 1 or 0 signifies a cow with udder score 5 or <5. For each cow an overall udder and teat score was calculated as the sum of these two values. The above score is based on teats >35 mm being associated with calf morbidity/mortality (Frisch, 1982) and the association between low-slung udders and increased time for calf to suckle (Ventorp and Michanek, 1992).

Based on mating management that targeted first mating of heifers at around 2 years of age, the first opportunity of calving is at 3 years of age. Therefore, the number of calving opportunities for a dam was calculated as the age of the dam in years, minus 2.

Fordyce *et al.* (2015) used urea space as a percentage of live-weight to estimate percentage body water in calves (w/v), i.e., hydration status. Fordyce *et al.* (2015) reported several regression equations to predict the outcome variable of hydration status. Data from the current study were fed into the regression equations to predict hydration status. Discrete indicator measures of hydration status included eye-lid tenting and eye ball recession. To avoid extrapolation when predicting percentage body water from published regression equations (Fordyce *et al.*, 2015), only data within the ranges of Fordyce *et al.* (2015) were used. For regression equations where oral temperature was used as an independent variable, the published adjustment for ambient temperature of Fordyce *et al.* (2015).

Initial graphing of live-weight over the first week of life indicated two growth profiles. Profile 1 calves appeared to have a consistent rate of live-weight gain through neonatal life. Profile 2 calves did not gain significant live-weight until day 2-3 of life, and thereafter appeared to grow at similar rates to profile 1 calves. Regression equations were fitted to explain each calf's increase in live weight from birth as a function of calf day of life. Discriminant analysis was conducted to determine likelihood of calves correctly being assigned to significantly different groups, based on increase in live weight between birth and day 3.

Daily change in live weight, plasma total protein and plasma globulin (indicator of colostrum intake) were used as outcome variables for calf milk uptake. To assess effects of measured variables to calf milk uptake, two-tailed t-tests were conducted for data measured during the first 3 days of calf life, to allow the expression of differences between calf groups at the stage where the groups varied in milk uptake. Measures investigated included maternal protectiveness, udder conformation and teat conformation. These analyses excluded the one dehydrating calf. Data were assessed as normally distributed with a Shapiro-Wilk test (α=0.05), including live-weight gain from birth, within each growth profile type and day of calf life. Therefore assumptions of normality were tested for group by day of life comparisons (Figure 1). Bartlett's test was used to confirm equality of variances (α=0.05). Where the above assumptions of the t-test were not satisfied, the Mann-Whitney U test was used. The Fisher's exact test was used to compare calf growth types, for all binary outcome variables, which are indicated in Table 1 by "(0/1)".

Analyses were conducted using Microsoft Excel version 14.0.7177.5000 and R version 0.99.879. Graphing was completed using the ggplot2 Package (Wickham, 2009) within R version 0.99.879.

3.3 Results

General observations of study calves through neonatal life indicate that all study calves except one were healthy and were exhibiting normal suckling activity. This is reflected by calf vigour scores and para-lumbar fossa distension scores (Figures 2 and 3).

One calf experienced dehydration, secondary to a dam with large, difficult to suckle teats. This dam was in moderate body condition with a very large and fully distended udder. Calf vigour score and para-lumbar fossa distension score appeared to decrease for the dehydrating calf, relative to well hydrated calves (Figures 2 and 3). The dehydration of this calf was reflected in its weight loss during neonatal life.

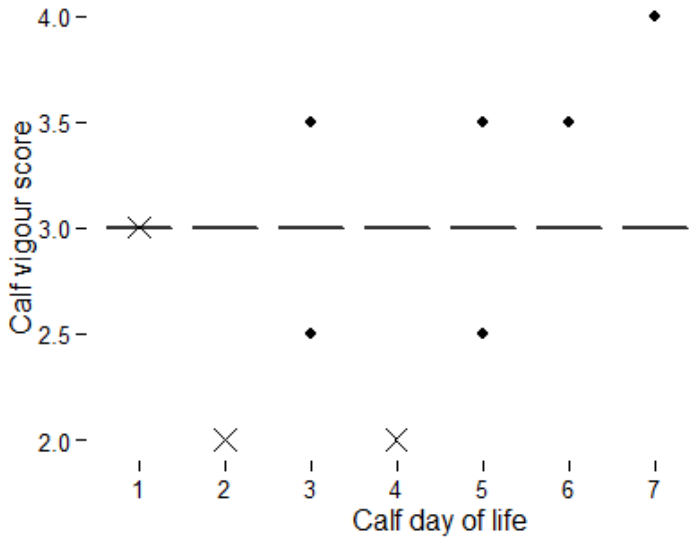

Figure 2. Calf vigour scores during neonatal life. Box and whisker plots=euhydrated calves, crosses=one dehydrating calf.

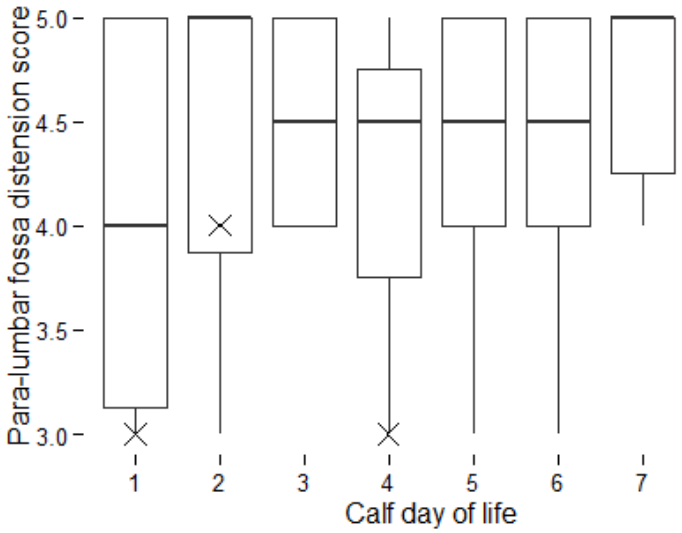

Figure 3. Calf para-lumbar distension scores during neonatal life. Box and whisker plots=euhydrated calves, crosses=one dehydrating calf.

Discriminate analysis of increase in calf live weight from birth separated calves into two groups of six. The dehydrating calf was not included in this analysis. For this analysis, the greatest difference in increase in live weight from birth between groups occurred at day 3 of life (Figure 1), where 0.66 of variation in cumulative increase in live weight between birth and day 3 of life was explained by calf grouping. Calves in the high and low group are denoted as rapid and delayed with cumulative live-weight gains from birth at day 3 of life being 3.12±2.05 and 0.28±0.54 kg, respectively.

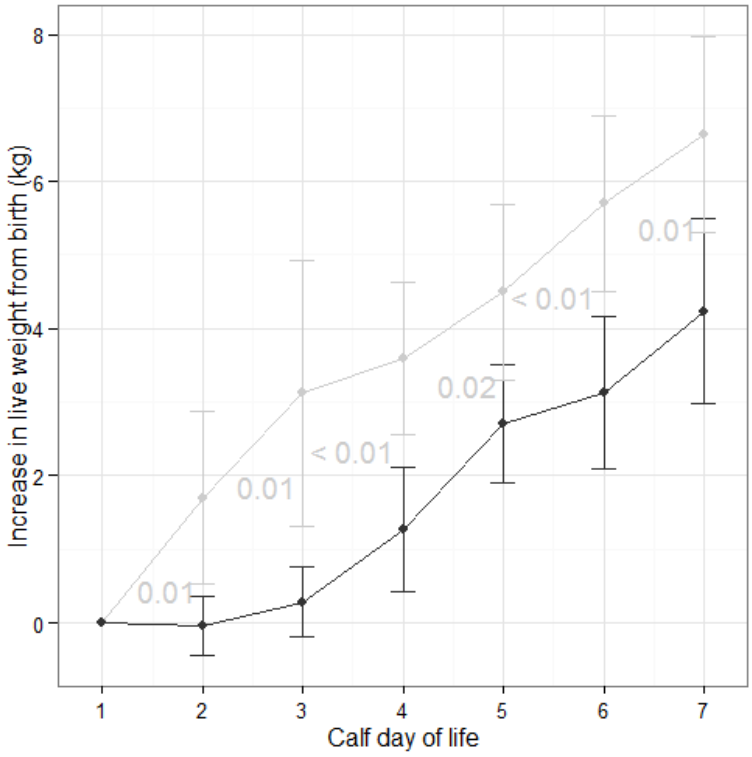

Figure 4. Line plot of raw data for mean (± 95% confidence intervals) increase in live weight from the first day of life for rapid (grey line, n = 6) and delayed (black line, n = 6) growth profile calves, with average birth weight of delayed and rapid groups of 30.2±2.9 and 29.6±2.9 (p =0.6). Labels within plot represent the p-value for difference between groups on a day.

Throughout neonatal life, the rapid group grew at 0.95 ± 0.24 kg/d, a rate that was not reached by the delayed group until day 4.2 ± 0.8 of life ($P < 0.01$). Delayed calves grew at an average of 0.32 ± 0.26 kg/d prior to achieving rapid growth. Early group trend of growth was represented by linear regression, and the delayed group was better represented by an exponential fit:

Linear regression of group 1: Increase in live weight from birth (kg) = -1.41 + 0.77 * calf day of life, p value = < 0.01, adjusted $R^2 = 0.92$

Exponential regression of group 2: Increase in live weight from birth = -1.53 + calf day of life^0.4

Summary statistics for the first 3 days of calf life are reported by calf group and are ordered as environmental, dam and calf variables and level of significance for differences between calf groups (Tables 1 and 2). During the first 3 days of calf life, rapid growth calves had higher plasma total protein concentration and plasma bicarbonate concentration, compared to delayed growth calves ($p<0.05$;Table 1). In addition, during the first 3 days post-partum, dams nursing rapid calves had decreased udder size ($p<0.05$) and visually-assessed higher udder distension ($p<0.01$) compared to dams with delayed growth calves (Table 1).

Table 1. Differential effects of environmental, calf and dam variables on growth group in 12 calves during days 1-3 of life

	Delayed group		**Rapid group**		**Difference between groups**
	Mean	95% confidence error margin	Mean	95% confidence error margin	p-value
Environment					
Ambient temperature (^{o}C)	27.6	1.9	28.2	1.9	0.68
Calf					
Growth rate (kg/d)	0.6	0.7	1.3	0.3	0.05
Oral temperature (^{o}C)	38.7	0.2	39.1	0.3	0.06
Calf increase in live-weight from birth (kg)	0.6	0.7	1.0	0.6	0.07
Front leg length (mm)	49.2	0.7	48.0	1.1	0.09
Rectal temperature (^{o}C)	39.2	0.2	39.5	0.2	0.09
Eyelid tenting time (sec)	1.1	0.1	0.9	0.2	0.16
Suckling response (1-5)	2.3	1.0	1.4	1.1	0.23
Para-lumbar fossa distension score (1-5)	4.3	0.3	4.6	0.4	0.23
Back leg length (mm)	72.5	0.7	71.8	1.4	0.37
Eyeball recession (mm)	0.1	0.1	0.0		0.43
Mucus membrane moistness (1-5)	5.0	0.1	5.0		0.43
Birth weight (kg)	30.2	1.2	29.6	1.5	0.49
Calf bunting (0/1)	0.1	0.2	0.0		0.51
Calf persistence to suckle (0/1)	0.3	0.2	0.1	0.2	0.62
Calf absolute live weight (kg)	30.5	1.3	30.9	1.8	0.8
Day of study at calf enrolment	4.4	4.1	6.8	4.4	0.81
Girth (mm)	67.8	1.4	67.8	1.7	0.98
Calf vigour score (1-5)	3.0	0.1	3.0		1.00
Milk residue on muzzle (0/1)	0.1	0.1	0.0		1.00

	Delayed group		Rapid group		Difference between groups
	Mean	95% confidence error margin	Mean	95% confidence error margin	p-value
Calf plasma variables					
HCO_3 (mmol/L)	14.6	1.4	16.9	1.1	0.02
Total protein (g/L)	75.8	6.5	87.4	8.2	0.04
Globulin (g/L)	50.3	7.7	62.9	9.6	0.06
Glucose (mmol/L)	6.9	0.8	8.1	0.8	0.08
Lactate (mmol/L)	5.1	1.2	3.7	0.7	0.19
Chloride (mmol/L)	99.5	1.2	98.3	1.3	0.24
Anion Gap (mmol/L)	29.1	1.5	28.1	1.5	0.34
Albumin (g/L)	25.1	0.9	24.5	1.5	0.45
Potassium (mmol/L)	5.8	0.3	5.9	0.5	0.51
Plasma urea (mmol/L)	2.7	0.6	2.5	1.0	0.65
Sodium (mmol/L)	137.5	1.2	137.2	1.6	0.72
Urea space variables					
Urea Space (L)	24.5	4.0	33.2	15.7	0.26
Urea Space as % of live weight (L/kg*100)	80.5	12.7	102.9	40.2	0.27
Dam					
Visually assessed udder distension score (1-5)	4.4	0.4	3.3	0.4	0.00
Udder size score (1-5)	3.5	0.4	2.7	0.4	0.01
Palpation assessed udder distension score (1-5)	3.2	0.7	3.9	0.6	0.08
Body condition score (1-5)	2.7	0.2	2.9	0.3	0.11
Dam number of calving opportunities	3.3	0.95	2.4	0.5	0.12
Poor udder or teat conformation	0.5	0.4	0.2	0.3	0.28

	Delayed group		Rapid group		Difference between groups
	Mean	95% confidence error margin	Mean	95% confidence error margin	p-value
score (0-2)					
Maternal protectiveness score (1-5)	1.5	0.2	1.7	0.4	0.43
Median teat width (mm)	30.8	4.2	30.6	2.0	0.44
Udder poor conformation (0/1)	0.1	0.2	0.0		0.5
Milk residue on udder (0/1)	0.1	0.1	0.0		0.52
Udder suspension score (1-5)	4.9	0.2	4.9	0.2	0.75
Poor teat conformation (0/1)	0.3	0.2	0.2	0.3	1
Dam bellowing (0/1)	0.0		0.0		A
Dam kicking calf (0/1)	0.0		0.0		A

A: p-value was not calculated, given no dam bellowing activity and no occurrence of dam kicking calf during observations.

Table 2. Prediction of hydration status (body water volume as percentage of live weight in 12 calves during days 1-3 of life

	Delayed group		Rapid group		Difference between groups
Univariable predictors	Mean	95% confidence error margin	Mean	95% confidence error margin	p-value
Live weight/hind leg length (kg/mm)	67.1	1.7	68.2	1.8	0.27
Oral mucus moistness score (1-5)	67.7	0.6	68.0	0.0	0.34
Eyeball recession (mm)	69.2	0.3	69.4	0.0	0.34
Eyelid tenting time (sec)	70.4	0.0	70.4	0.5	1.00
Multivariable predictors[A]					
Plasma sodium (mmol/L)	69.4	1.9	71.0	1.7	0.23

Eyelid tenting time (sec)	66.6	2.3	68.6	2.6	0.27
Vigour/alertness score (1 – 5)	60.3	2.8	62.7	3.3	0.27

A: Percentage body water predicted using multivariable regression equations (Fordyce *et al.*, 2015) that use independent variables of weight/hind leg length (kg/mm), girth/hind leg length (mm/mm), adjusted oral temperature (^{o}C) and a fourth variable as listed

Differences in growth between calf groups were reflected in the trends of plasma total protein, globulin and glucose concentrations (Figure 5). These differences between the growth type groups were highest within the first 2 days of neonatal life (Figure 5). Throughout neonatal life, calf groups differed in plasma total protein concentration at day 1 ($p<0.05$), though this difference is total plasma protein ceased to differ between groups by day 2 of life ($p=0.23$). Calf groups differed in plasma glucose concentration at day 1 of life ($p<0.05$), with this ceasing at day 2 ($p=0.09$). Despite clear trends suggesting calf groups differed in plasma globulin concentration, the difference was not significant at any day of neonatal life. Despite the association of calf oral temperature and growth profile approaching significance ($p=0.06$) over the first 3 days of life (Table 1), calf groups only differed in oral temperature at day 3 of life, with rapid and delayed groups having oral temperatures of 39.3±0.36 ^{O}C and 38.8±0.34 ^{O}C ($p<0.05$, figure 5E).

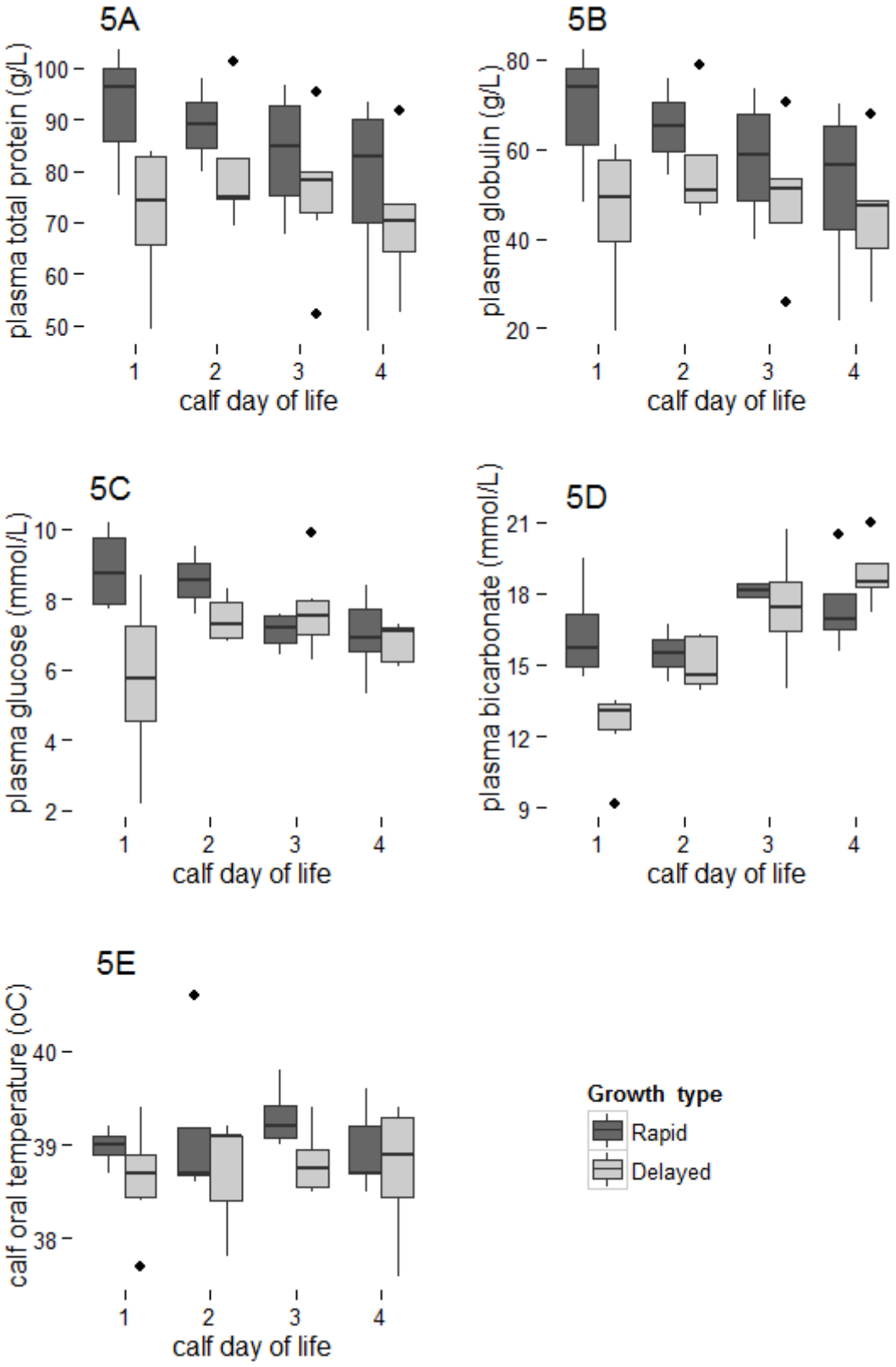

Figure 5. Box and whisker plots each presenting variation in indicator variables for calf milk uptake of calf plasma protein, plasma globulin and plasma glucose, over calf day of life, for the rapid and delayed growth calf groups.

Similarly, through the first week post-partum, cows nursing delayed growth calves tended to have higher udder size than those nursing rapid growth calves, with significant differences occuring between groups only at days 1 and 4 of calf life (Figure 6). Although there was a trend where visually-assessed udder distension was higher in dams of delayed growth calves through the first 3 days of life, the difference was only significant at day 3 (Figure 7). An opposite trend occurred for palpation-assessed udder distension, where dams of rapid calves tended to have higher palpation-assessed udder distension compared with dams of delayed calves (p=0.08), especially at day 3 of calf life. Only 2-3 measurements were taken for palpation assessed udder distension in each group.

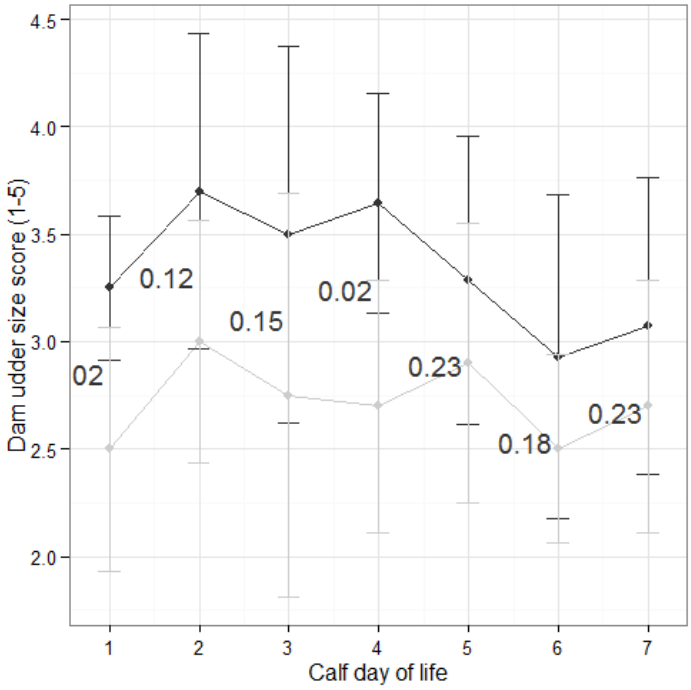

Figure 6. Dam udder size score during the first week post-partum. Black line=Delayed growth type calves, grey line=rapid growth calves.

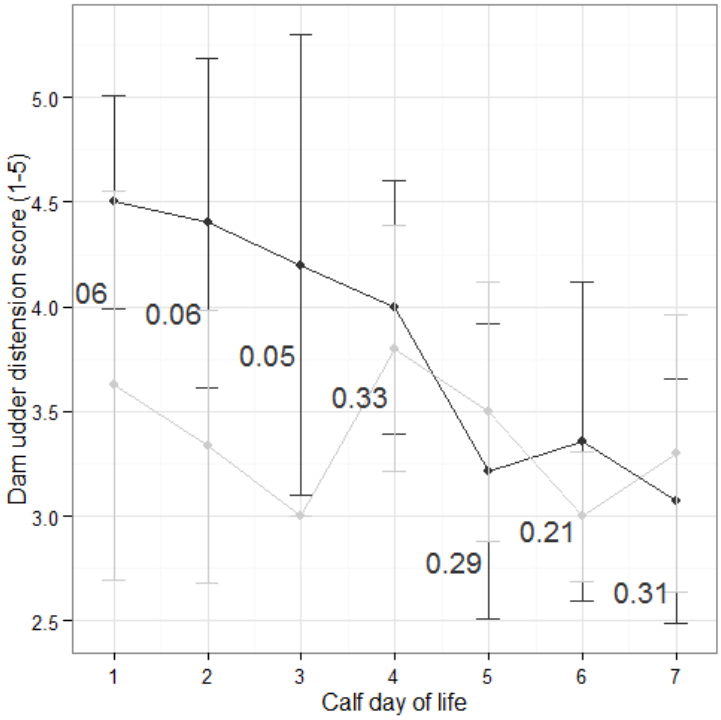

Figure 7. Visually-assessed udder distension during the first week post-partum. Black line=Delayed growth type calves, grey line=rapid growth calves.

3.4 Discussion

Variation in milk uptake

This study has shown that approximately half of a Brahman calf cohort experienced very little growth until an average of 4 days post-calving, under well-managed conditions in the dry tropics. Calf growth is determined by solid and fluid accruing as tissue growth and is primarily a function of milk intake (Totusek *et al.*, 1973, Black, 1982, Castells *et al.*, 2014, Bartle *et al.*, 1982, Montsma, 1960). Therefore clear differences in growth within the first 3 days of life are likely explained by variation in milk uptake. In addition, evidence of increased milk consumption in the early group, compared to the delayed group is indicated by measures of calf plasma concentrations of bicarbonate, total protein, globulin and glucose, for reasons discussed below.

At day one of calf life, compared to the delayed group, the early group had increased plasma concentrations of bicarbonate ($p<0.02$), and tended to have increased lactate ($p=0.19$). This is consistent with a study on infants demonstrating that milk consumption leads to net base production (Chan *et al.*, 1980). The non-significance of difference in lactate between calf groups at day one of life is likely due to lack of measures (early group n=3, delayed group=6), as with substitution of mean delayed group lactate to theoretically add measures only required two additional measures to achieve significance ($p<0.05$). Plasma bicarbonate ceased to differ between groups by day 2 of calf

life. This is consistent with the results of Moore (1969) suggesting that normally born, newborn calves are in a state of acidosis at birth, and between birth and 24 hours of birth, plasma lactate decreases and plasma bicarbonate concentration tends to increase. This is consistent with infants who are born in a state of metabolic acidosis, with blood bicarbonate increasing between birth and 24 hours of life, and corresponding decreases in lactate (Koch and Wendel, 1968). Therefore with homeostatic decrease of acidosis by day 2 of life in calves, differences in bicarbonate and lactate are likely only sensitive to level of milk consumption for calves less than 24 hours of age.

At day one of calf life, early and delayed calves had plasma protein concentrations of 91.7±14.9 and 71.1±14.3 ($p<0.05$). This suggests a larger volume was consumed by the early calf group, and is consistent with the study of Danijela *et al.* (2002), where calves fed 3 or 1.5 L of dams colostrum at 2 hours post-partum had serum total protein concentrations of 54.7±10.2 and 42.7 ±10.6 at 4 hours post-partum. Although calf groups did not significantly differ in plasma total protein concentration at day two of life ($p=0.23$), a biological difference may have occurred with lack of significance due to low number of measures (early group n=2, delayed group n=5); where significance was achieved ($p<0.05$) by including group means as theoretical values where only seven observations in each group were required.

Plasma globulin concentration tended to be higher for early than delayed calves, especially during the first three days of calf life. This indicates higher colostrum uptake in the early group compared to the delayed group, as colostrum consumption, and increases in plasma total protein and globulin concentrations occurred within 24 hours of feeding (Zanker *et al.*, 2000). In addition, newborn calves are born in a practically agammaglobulinaemic state (Cabral *et al.*, 2012). Trend of difference in plasma globulin concentration between calf groups steadily diminishes over neonatal life. This is consistent with studies that describe decreasing impact of colostrum uptake on plasma globulin concentrations, due to decreasing ability of calf to absorb globulin through neonatal life (Rajala and Castren, 1995) and decreasing colostral globulin concentrations during the 48 hours days post-partum (Crowther and Raistrick, 1916). Therefore plasma globulin is a biologically relevant measure of colostrum uptake in the first 48 hours of life, and especially the first 24 hours of life. Although plasma globulin concentrations did not significantly differ between groups on days one and two of life, a biological relationship may occur, which is not expressed due to low number of measures, (day one of life, early n=4 and delayed n=5; n= day two of life, early n=2 and delayed n=5). With theoretical addition of values, significance was achieved at day one ($p<0.05$; n=8 per group) and day two ($p<0.05$; n=7 per group). The study of Danijela *et al.* (2002), which showed differences in total protein, had 8 calves in each high and low colostrum group. Therefore the use of plasma globulin as a measure of milk uptake may be verified with further experimentation.

Colostral total immunoglobulin concentration was not associated with calf serum total Ig concentration (Rajala and Castren, 1995). Similarly, correlation between cow colostrum IgG_1 concentration and calf serum IgG_1 concentration was low (Norman and Hohenboken, 1981). Despite this, in neonatal calves, increased volume of colostrum uptake of calves was correlated with total protein and globulin concentrations (Kühne *et al.*, 2000). Therefore calf plasma globulin concentration may reflect volume of colostrum uptake more than colostral globulin concentration.

Plasma glucose was higher in the early group than the delayed group at day one of life ($p<0.05$), with tendency for this difference at day 2 ($p=0.09$), despite only 2 and 5 measures for the early and delayed groups on this day. This is consistent with the study of Zanker *et al.* (2000), where increase in calf plasma glucose concentrations generally occurred two hours after colostrum feeding.

No cows of the current study were in their first lactation, and the early and delayed calf groups were of dams at their 2.4±0.5 and 3.3±0.95 calving opportunity ($p=0.12$). In dairy cows, those of first parity had lower IgG concentration than parities 3, 4, and 5+, which Conneely *et al.* (2013) had attributed to increased age and therefore time exposed to pathogens. Conneely *et al.* (2013) completed this analysis as a multivariable model, to account for differences in colostral volume. Similarly, the greatest difference in parities for volume of colostrum produced exists between first and higher lactation cows (Conneely *et al.*, 2013). Given that dams of the early calves tended to be of lower parity, parity is likely not an important variable in explaining differences in calf plasma globulin concentrations between groups. In addition, cows were under the same environmental and management conditions prior to experimentation, and therefore season is unlikely to be an important variable explaining difference in plasma globulin concentration between calf groups.

Birth weight and calf vigour score did not differ between early and delayed growth calves. This suggests that a foetal nutritional restriction and consequent issues reported in calves including low muscle fibre development (Funston *et al.*, 2010) and possibly calf metabolic ability to use milk was not limiting factor growth in this study.

Measuring milk uptake

Calculated urea space as percentage of live weight values were unrealistic and deemed inaccurate, given previous reports of calf percentage body water being approximately 74% (Haigh *et al.*, 1920, Dalton, 1964, Fordyce *et al.*, 2015). This is consistent with a report on healthy foals, where the urea space method had a higher error variation than the tritiated water method, where the tritiated water method was a higher cost, and more labour intensive technique (Geerken *et al.*,

1988b). It was found that for all extreme urea space as a percentage of live weight values (i.e., <52% or >215%), there were obscure increases in plasma urea values (7.0 ± 7.6 mmol/L and 0.9 ± 0 mmol/L, respectively), for the 12 minutes after urea infusion. For urea space as percentage of live weight values of 73 – 105, increase in plasma urea concentration was 2.5 ± 0.2 mmol/L. Therefore the increase in plasma urea values that were far lower or far higher resulted in urea space as a percentage of live weight values that were either unrealistically inflated or low, respectively. The source of error is not clear. For each particular calf that had an extreme urea space as a percentage of live weight value, urea space as a percentage of live weight and plasma increase in urea values tended to be similar for days prior to and after when the extreme values occurred. In a theoretical exercise, where 2.44 was substituted for the increase in plasma urea for the extreme values, all values became well within the range of urea space as a percentage of live weight values in the analysed dataset and the overall average and standard deviation closely matched that of the analysed dataset.

Dalton (1964) discussed how error in urea space as a percentage of live weight may be due to error in live weight measurement. Specifically, low gut fill in a calf can inflate the calculated urea space as a percentage live weight, given a decrease in the denominator and no change in the numerator (Dalton, 1964). For this study, a theoretical data manipulation was conducted to determine if error in live weight (e.g., that of gut fill, bladder fill, or error in scale readout) were the source of error, by theoretically adjusting the live weight 1.6 kg higher or lower, and thus theoretically adjusting the urea dose infused to the calf. For a calf of 32 kg and calculated urea space as a percentage of live weight of 86%, theoretically adjusting weight up or down 1.6kg resulted in respective urea space as percentage of live weight values of 90% and 81%.

On average, the percentage body water values predicted using published regression equations (Table 2) appeared comparable with reported values of calf body water content, though when plotted on a per calf basis, there appears to be a problem where percentage body water is increasing with live weight gain from birth. This may be explained by issue with the prediction equation being not applicable across genotypes.

Although it is difficult to determine actual trends of hydration using practical field measures general observations in this study provide evidence that well hydrated calves grow at different rates (Table 4.4) and exhibit various growth curves.

Although the dehydrating calf demonstrates how measures of calf vigour score and para-lumbar fossa distension imprecisely quantify dehydration, as in Fordyce *et al.* (2015), these measures may still prove useful in quantifying the relative frequency of neonatal calves that experience dehydration, in large herds under field conditions.

In contrast to the results of Fordyce *et al.* (2015), these calves in neither group were not milk deprived to the extent that hypernatraemic dehydration occurred, and therefore plasma sodium and urea are not a useful measure for these calves. Despite this, this study demonstrates how live weight, globulin, total protein and glucose can assist in quantifying milk uptake.

Implications for industry

This study demonstrates variation in milk uptake between calves. If this variation in milk uptake exists in breeding herds under conditions of higher nutritional and environmental stress, then cows with the lowest milk production in the 3-days post-partum are likely to have calves at high risk of dehydration mediated mortality. Milk-deprived tropically adapted neonatal calves can lose 20% of body water within 2-3 days, at which point they lose ability to suckle (Fordyce *et al.*, 2015). For a calf of birth weight ~30 kg, this equates to approximate body water loss of 4.5 L, equating to approximately 5.22 L of milk (at 85% water). The lower level of milk production in this study is likely at calf maintenance levels, but could be much lower in other herds.

3.5 Conclusion

A high frequency of delayed growth in neonatal Brahman calves occurred, with evidence that this was related to delayed lactation. Plasma total protein was lower in calves with delayed growth, providing additional evidence of low milk uptake. If reasons for low milk yield during the first 3-days post-partum can be clarified, then interventions to reduce neonatal calf mortality may be developed.

The parallel trends of live-weight and plasma parameters verify each other as indicators of milk delivery to calves, as both were significantly reduced in the delayed calf group, compared to the rapid calf group.

Acknowledgement

The staff at Spyglass Beef Research Facility are gratefully acknowledged for their assistance.

4. Investigation into risk factors for calf mortality in two tropically-adapted northern QLD beef herds

4.0 Abstract

The aims of this study were to describe the occurrence of risk factors for calf mortality and to quantify the incidence risk of neonatal mortality for each risk factor. The incidence risk of neonatal mortality in the southern forest of Queensland and northern forest of Queensland was 3.1% (21/683 live births) and 4.7% (14/297 live births), respectively. Some risk factors and the percentage exposure of calves to high risk for mortality included: dam body condition score ≤2 (8%), birth weight ≤28 kg, low birth vigour (2%), large udder size (1%), at least two large teats (2%), poor maternal protectiveness (5%). Temperature-humidity peaks of similar magnitude occurred during early pregnancy at both sites and for around calving in the northern forest, with a lower peak around calving in the southern forest. The higher incidence risk of neonatal mortality in the northern forest than the southern forest may be at least partly explained by the higher proportion of low birth weight calves in the northern forest. The majority of neonatal calves were observed to be generally well-hydrated and strong. Given the low proportion of cows in poor body condition and of neonatal mortality, nutritional management limited the opportunity for dehydration-mediated neonatal mortality. Low calf mortality may be achieved in any country type, provided that a low proportion of calves are exposed to high risk for mortality.

4.1 Introduction

It is estimated that approximately one million calves die in Australia every year (Fordyce, 2017). Within cows that achieve pregnancy across northern Australia, median incidence risk of foetal and calf mortality ranges from 5% (for first lactation cows in the southern forest) to 16% (for heifers in the northern forest; McGowan *et al.*, 2014). A 2.7% increase in annual incidence risk of foetal and calf survival was associated with an increase in live-weight production of 10 kg/cow(McGowan *et al.*, 2014). The majority of calf mortalities remain unexplained (Bunter *et al.*, 2013, McGowan *et al.*, 2014).

McGowan *et al.* (2014) monitored 78 000 breeding cows, where interquartile ranges for incidence risk of foetal and calf mortality were between 5% and 14%.This indicates considerable variation in foetal and calf mortality and possible opportunity to decrease the incidence risk of foetal calf mortality in many breeding herds. Neonatal mortality (i.e., mortality in the first week of life) accounted for 67% (Bunter *et al.*, 2013) and 43.8%[3](Holroyd, 1987) of foetal and calf

[3] Although the incidence risk of neonatal calf mortality was not reported in Holroyd (1987), the authors reported a count of pregnancies studied (3285), total losses between pregnancy diagnosis and weaning (516), calf mortality within 48 hours of life (170) and calf mortality between hour 48 and day 7 of life (56).

mortalities. Therefore, the reasons explaining variation in incidence risk of neonatal mortality may also drive much of the reported variation the in incidence risk (termed incidence hereafter) of foetal and calf mortality.

The reasons for the high risk of mortality during neonatal life are poorly understood (Bunter *et al.*, 2013, Holroyd *et al.*, 2005). McGowan *et al.* (2014) reported that compared to cows on higher levels of nutrition, those on low phosphorus (<500 mg/kg ME) had an incidence of foetal and calf mortality that was 3.4 percentage points higher; and those on poor quality pasture during the dry season (pasture crude protein: dry matter digestibility ratio <0.125) had an incidence of foetal and calf mortality that was 4 percentage points higher. McGowan *et al.* (2014) also reported that a temperature-humidity index of greater than 79 for at least 14 days around calving was associated with an increase in incidence of foetal and calf mortality that ranged from 3.5% in the southern forest country to 7% in the Northern Downs. Bunter *et al.* (2013) reported that neonatal calf mortality was associated with cohort. Bunter *et al.* (2013) also reported that cow age impacted on neonatal mortality, where, for example, neonatal calves of second lactation cows had 3.62 greater odds of mortality than those of first lactation cows. Bunter *et al.* (2013) also reported that calves born to a dam with a large udder had a 1.89 greater odds for neonatal mortality than those with a moderately sized udder. Bunter and Johnston (2014) reported that the heritability of calf mortality (between birth and weaning) for Brahmans and tropical composites as 0.09±0.02 and 0.02±0.01, respectively. Bunter *et al.* (2013) also reported that calves of birth weight <29 kg had a 2.12 higher odds of neonatal mortality than those of birth weight >39 kg. Birth weight has been associated with breed (Bunter *et al.*, 2013), dam nutrition in the last trimester of pregnancy (Fordyce *et al.*, 1993), calf vigour and survival to weaning (Schmidek *et al.*, 2013). Level of protein in the cow diet during gestation affects calf vigour (Bull *et al.*, 1974). Most of the above risk factors for neonatal mortality could clearly impact on milk delivery to neonatal calves and may mediate their impact on mortality by either low calf ability to suckle, low dam milk production, or dam mortality(Fordyce *et al.*, 2015).

Although foetal and calf mortality is associated with general nutritional and environmental stress (McGowan *et al.*, 2014, Wythe, 1970), practical management practice that could be modified to reduce their impact remains challenging without a specific understanding of the mechanisms by which these stresses lead to calf mortality. The effectiveness of such modification can crudely be assessed by the extent that calf mortality is reduced. Therefore, the suitability of a management practice change will dependent on: the degree to which it may be modified or manipulated, the

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Therefore the incidence risk of neonatal mortality was calculated as percentage of total foetal and calf mortalities that occur during the first week of life. Holroyd also reported a pre-natal mortality in 3.5% of pregnancies.

strength of its association with calf mortality and proportion of calves affected within the at risk population.

The aims of this study were to describe the occurrence of risk factors previously associated with calf mortality and to quantify the incidence of neonatal mortality for each risk factor in two low-mortality tropically-adapted herds within the northern and southern forest regions of northern Australia where typical calf loss rates can be high (McGowan *et al*., 2014). A reason for clarifying the proportion of calves exposed to high risk for calf mortality in low-mortality situations was to consider interactions between risk factors. Such interactions may explain why a risk factor may be impacting on calf mortality in one situation but not another.

The main hypothesis is that in situations where incidence of neonatal mortality does not exceed 5%, there is also a low frequency of risk factors that have previously been associated with various measurements of calf loss, e.g., foetal and calf loss. Acceptance of this hypothesis would indicate that reduced incidence of neonatal calf mortality could be achieved in other situations, provided the frequency of risk factors are reduced.

4.2 Materials and Methods

Ethics approval

The data used in this study was collected as part of the "Repronomics" project (MLA project B.NBP.0759) which was conducted with approval from the Animal Ethics Committee of the Queensland Department of Agriculture, Forestry and Fisheries.

Site environments

The study was conducted at two sites situated in highly variable climates of Queensland, Australia. Brian Pastures Beef Research Facility is situated in the southern forest land type of the sub-tropics (-25.65^{o}S, 151.75^{o}E). Spyglass Beef Research Facility is situated in the northern forest land type of the dry tropics (-19.49^{o}S, 145.68^{o}E). The targeted calving period occurred from September 2015 to February 2016.

A long term climate summary (Table 1) revealed that summer temperatures peak in January at Brian Pastures and in December at Spyglass. Lowest winter maximum temperatures occurred in July at both sites.

Table 1. Long term climate summary for research sites

			Summer		Winter	
	Average annual rainfall (mm)	Peak rainfall period	Maximum daily temperature (^{o}C)	Minimum daily temperature (^{o}C)	Maximum daily temperature (^{o}C)	Minimum daily temperature (^{o}C)
Brian Pastures	704	October - March	33	20	22	7
Spyglass	660	November - March	34.8	21.2	24.5	11

A rainfall and temperature figures based on long-term monthly averages calculated using measurements started at in 1882 at Charters Towers Post office and in 1968 at Brian Pastures Research Facility (130 mm; Bureau of Meteorology, 2016b).

Brian Pastures paddocks were mostly cleared of trees. Predominant pasture species at Brian Pastures included black spear grass (*Heteropogon contortus*), forest blue grass (*Bothriochloa bladhii*) and wire grasses (*Aristida spp.*). Calving paddocks at Brian Pastures were on average approximately 25 hectares, with one to three watering troughs per paddock. One calving paddock at Brian Pastures contained a dam as the single watering point.

Spyglass paddock types included native pasture used for the period from mating to mid-pregnancy and improved pasture used for fixed time artificial insemination programs and calving. Native pasture paddocks were approximately 1400 hectares and included native trees. Improved pasture paddocks were approximately 300 hectares and were mostly cleared of trees. In the improved pasture paddocks, the predominant pasture species was buffel grass (*Cenchrus ciliaris*). In the native pasture paddocks, the predominant species were black spear grass (*Heteropogon contortus*), kangaroo grass (*Themeda trianda*) and wire grasses (*Aristida spp*). Calving paddocks generally had one or two troughs for watering points.

Animals and management

The cattle breeds studied included Brahman and Droughtmaster at Spyglass with the additional breed of Santa Gertrudis at Brian Pastures. For the purposes of this study, both Droughtmaster and Santa Gertrudis are categorised as tropical composite breeds. All cows in the study were born off site, except those cows born in late 2013 which experienced their first lactation during this study. Externally sourced breeding females were from the northern forest (Swan's Lagoon) and central forest (Belmont) research sites for Spyglass and from a northern downs research site (Toorak) for Brian Pastures.

The mating periods were 14/January/2015 to 08/April/2015 at Spyglass and 25/November/2014 to 11/February/2015 at Brian Pastures. In the mating paddocks at each site,

nulliparous heifers were first exposed to bulls at approximately two years of age, along with first-lactation cows and their calves at approximately 3 years of age. Mating groups included approximately 200 breeding females per paddock. Separate multiple-sire mating paddocks were used for each breed. During the mating period at each site, multiparous cows (parity >1) were provided two opportunities to achieve pregnancy by fixed time artificial insemination. At approximately a month after the first artificial insemination of cows, pregnancy diagnosis was conducted on these cows and they were re-enrolled in the second artificial insemination program if not pregnant. This was followed by exposure to bulls. The multiparous cows were a mixture of those cows that had, and had not, raised a calf in the previous year and included some cows from the second-lactation group of the previous year. At each site, pregnant animals of all breeds were managed in the same paddock.

Weaning occurred at a final pregnancy diagnosis when calf age averaged approximately seven months. At each research site, approximately one month prior to the commencement of calving, pregnant cows were moved to separate calving paddocks based on predicted dates of calving (calculated with foetal age data collected at pregnancy diagnosis). The size of the calving groups varied with paddock size and ranged from approximately 15 to 70 cows at Spyglass to groups of 50 to 100 cows at Brian Pastures.

On the 11/August/2015, 294 pregnant cows were moved from Spyglass to Brian Pastures, due to poor season and pasture condition at Spyglass. The trucking occurred at 209 days after the start of the mating period at Spyglass and therefore cows were likely no longer than approximately 209 days into pregnancy. Cows trucked from Spyglass to Brian Pastures were managed on Brian Pastures, while the majority of cows from Brian Pastures were managed on an agistment property of similar country type near Brian Pastures.

Calving occurred between mid-October 2015 and late-January 2016 at Spyglass and between mid-September 2015 and early-February 2016 at Brian Pastures. Every two to four weeks during the calving period, cows in the calving paddocks were yarded to separate and relocate cows with calves to another paddock. The objective was to conserve pasture for cows that were either still gestating or with calves that were too young to move.

Supplementation and parasite control

At both sites, all cattle were vaccinated annually when cows were in mid-pregnancy. Vaccines targeted clostridial diseases and leptospirosis (7-in-1®, Zoetis), botulism (SingVac 1 Year®, Virbac) and pestivirus (Pestigard®, Zoetis). Bulls received vaccine to prevent campylobacterosis (Vibrovax®, Zoetis). During the year, breeding females received anti-parasite

treatment in response to the ecto-parasite load. Buffalo fly tags (PYthon ®, Y-Tex), and tick (*Ripicephalus microplus*) pour-on products (Acatak Duostar®, Novartis) were applied.

Cows had *ad libitum* access to available pasture within paddocks at each research site. There was a similar supplementation regime for all paddocks on a site.

Spyglass supplementation targeted an *ad libitum* intake (~2 kg/cow/day) of a fortified molasses supplement (including cracked corn and 8% urea) whenever pasture was in a state of senescence. *Ad libitum* molasses-urea lick blocks were also available at all times.

At Brian Pastures, during the period from mid-September to late-November 2015, cows were supplemented with a lick that contained 15% urea and 5% salt at a target intake rate of 300 g/cow/day.

Measurements

Measures recorded at birth. During calving periods, a team of two people checked cows daily throughout the period starting at 7:00 am and finishing at approximately 11:00 am. Each calf was weighed in a metal cradle using analogue scales (Kain Chung Scale Factory, KC-08, 100 kg). Birth weights were recorded to the nearest 0.5 kg. Subjective assessments recorded on a 5-point scale included:

Maternal protectiveness score at parturition (1 = dam absent, 2 = dam at distance and not attentive during calf capture, 3 = dam attentive to calf from distance during calf capture, 5 = dam extremely protective and close to calf during capture)

Calf vigour (1 = calf unable to stand, 2 = calf able to stand but very sluggish, 5 = calf highly vigorous and difficult to restrain)

Udder size (1=very small, 5=very large)

Size of each teat (1=very small, 5=very large)

Dam body condition score (1 = very poor, 2 = poor, 3 = moderate, 4 = forward, 5 = very fat) in third score increments

During the daily checks, any calf mortality and any evidence potentially explaining the mortality was noted. For example, if a calf was found dead with smooth unworn hooves, this was noted as evidence of a possible stillbirth or weak calf. As another example, the presence or absence of dam at a calf carcass was noted, to provide some evidence on maternal attentiveness.

Weather and pasture. Rainfall was measured at each site using rain gauges. Maximum daily temperature and humidity values at maximum daily temperature were calculated based on Data Drill, i.e., data interpolations from nearby weather stations (Bureau of Meteorology Data Drill)and the following **Equation 1** (Hahn *et al.*, 2009) was applied.

$$Temperature\ Humidity\ Index = 0.8 \times Temperature\ (℃) + [Percent\ Relative\ Humidity \times (Temperature\ (℃) - 14.4)] + 41.6$$

Data management and analyses

Neonatal mortality was calculated as the number of live-born calves dying within the first week of life divided by the total number of calves born in the same time period. For the purposes of this study, any calves that required assistance to nurse or were given milk replacer were considered mortalities. Incidence of neonatal mortality was calculated using the design effect to account for clustering within the two research sites, using the epiR package (Stevenson *et al.*, 2016) within R (R Core Team, 2014).

The following risk factors were considered in the analysis: total number of days where cows experienced temperature-humidity index of >79 during the first or second half of pregnancy, cow parity, dam body condition at calving, dam udder size, dam having at least two large teats, dam maternal protectiveness, calf birth weight, calving difficulty and calf vigour at birth.

A set of data cleaning and data preparation procedures were conducted before any analysis was conducted. The threshold for low calf birth weight was set at a birth weight below the 20th percentile. Not all teats of a particular cow are the same size. Therefore, cows were categorised on whether they had at least two score five (bottle) teats or not at calving. Early or late calving was defined as being born before or after 330 days from the start of the mating. Therefore cows mated at Brian Pastures were late if calved after 7/October/2016and cows mated at Spyglass were late if calved after 10/December/2015. A threshold was set to create a binary variable where pregnancies were either exposed to <15 or ≥15 total days of temperature-humidity index >79 during the first half (145 days) of pregnancy. This method was also used for the second half of pregnancy. Day zero of pregnancy was calculated as birth date minus 290 days, based on the average Brahman gestation length reported by Reynolds *et al.* (1980). This date was used for day 0 of pregnancy, where temperature-humidity index (THI) data are presented on a day of pregnancy basis (Table 3). Temperature-humidity index was estimated as a function of day in pregnancy by fitting general

additive models and was graphed, by research site, using the ggplot2 package (Wickham, 2009) within R (R Core Team, 2014).

The proportions of calves exposed to and not exposed to the high level of risk were tabulated. Within each risk factor level, the incidence estimates for neonatal mortality and their 95% confidence intervals were adjusted for clustering using the exact method and Fleiss procedure implemented using the epiR package (Stevenson *et al.*, 2016) within R. A calculated risk ratio of 'x' gives a value which is interpreted as: the incidence of neonatal mortality is 'x' times higher for those calves exposed to a given level of risk factor compared to those exposed to a 'reference' level of a risk factor. Risk ratios and their 95% confidence intervals were estimated using generalised linear models and robust standard errors estimated using the exact methods adjusted coefficient with the sandwich package (Zeileis, 2006) within R. Risk ratios were tested using the lmtest package (Zeileis and Hothorn, 2002) within R. The global-log-likelihood ratio statistic was used to compare a baseline model with the incidence of neonatal mortality only, with a model for predicting incidence of neonatal mortality with the risk factor. Wald test p-values were generated. The base R package was used for the above calculations (R Core Team, 2014).

4.3 Results

Monthly rainfall reflected the typical bimodal distribution of rainfall at Spyglass (Figure 1), where very limited rainfall was received between February 2015 and October 2015. The low rainfall limited any substantial pasture growth prior to the wet season. The rainfall at Brian Pastures followed the typical annual pattern but had an earlier start to the wet season than Spyglass (Figure 1). The total annual rainfall for 2015 at Brian Pastures and Spyglass was 823 and 257 mm, respectively. The long term averages for annual rainfall is 648.2 mm near Spyglass[2] and 703.5 mm at Brian Pastures[2] (Bureau of Meteorology, 2016a).

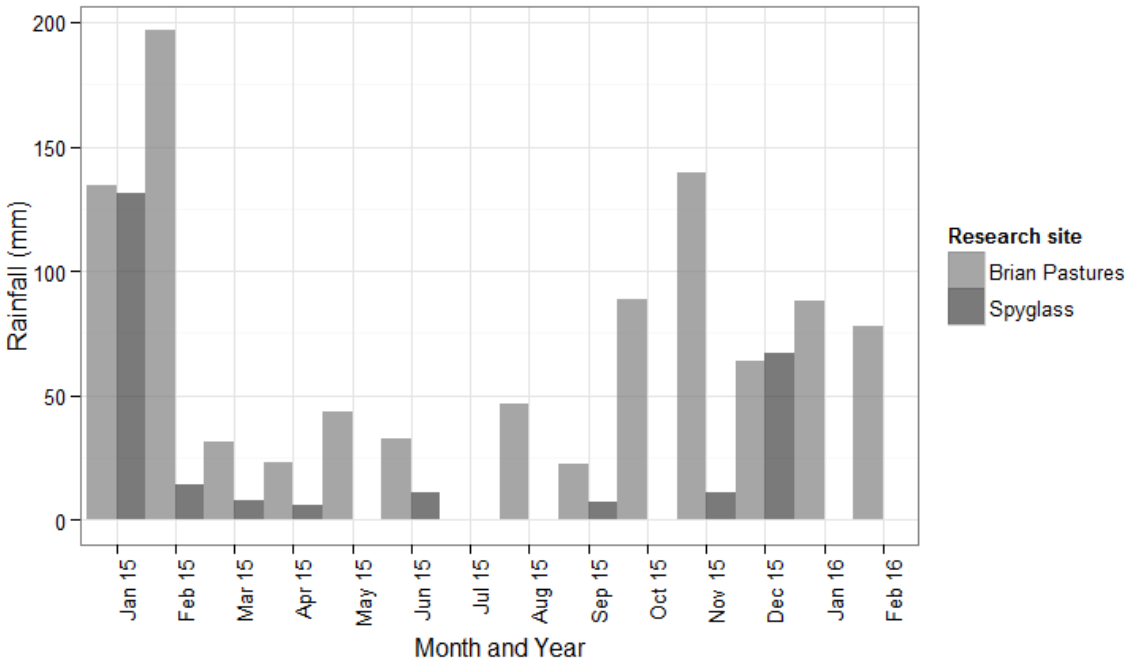
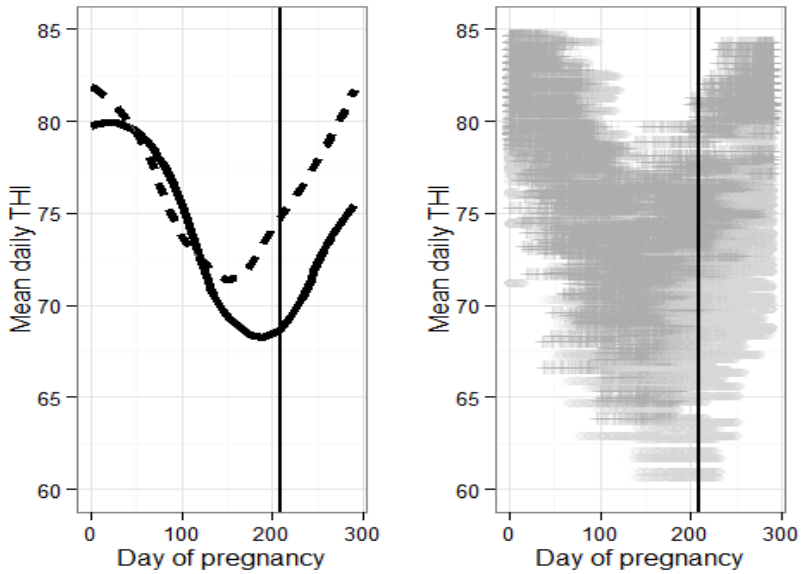


Figure 1. Rainfall at Brian Pastures and Spyglass during 2015 and early 2016[4]

At both sites, the peaks of temperature-humidity index generally occurred during early and late pregnancy (Figure 2). During late pregnancy, the peak of temperature humidity index tended to be lower at Brian Pastures than Spyglass (Figure 2).



2a. **2b.**

Figure 2a: Line plot of smoothed temperature-humidity index experienced by cows as a function of research station (297 cows at Spyglass and 389 cows at Brian Pastures) and days of gestation in the study period as calculated from birth dates. Smoothed lines were generated using general

[4]Long term rainfall averages for Spyglass are based on records from Charters Towers post office, which is approximately 110 km south of Spyglass

additive models implemented with general additive models with ggplot2 package in R. Solid lines=Brian Pastures, Broken lines=Spyglass. **2b**: raw data to demonstrate variation in temperature-humidity index. Light grey=Brian Pastures, Dark grey=Spyglass. Line at day 209 of pregnancy is the approximate stage at which some cows were moved from Spyglass to Brian Pastures. Cows moved from Brian Pastures to Spyglass mid-pregnancy (n= 294) were excluded, though would have experienced that same conditions at Spyglass cows to the left of the line and Brian Pastures cows to the right of the line.

During the first half of pregnancy, few cows were exposed to a temperature-humidity index of >79 for less than 15 days (Figure 3). During the second half of pregnancy, approximately 500 cows experienced a temperature-humidity index of >79 for less than 15 days (Figure 3).

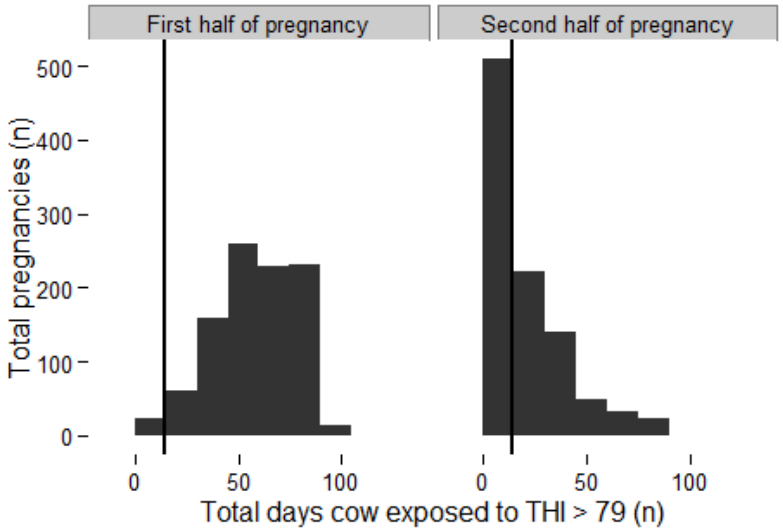


Figure 3. Distribution of pregnancies for total number of days where temperature-humidity index at maximum daily temperature exceeded 79. Vertical lines denote high-risk threshold for calf mortality also used in Table 4. n=977.

Despite the high at-risk days that individual cows were exposed to temperature-humidity index >79 in early pregnancy (Table 4), no cows experienced a temperature-humidity index of >79 for longer than six consecutive days throughout pregnancy (Figure 4).

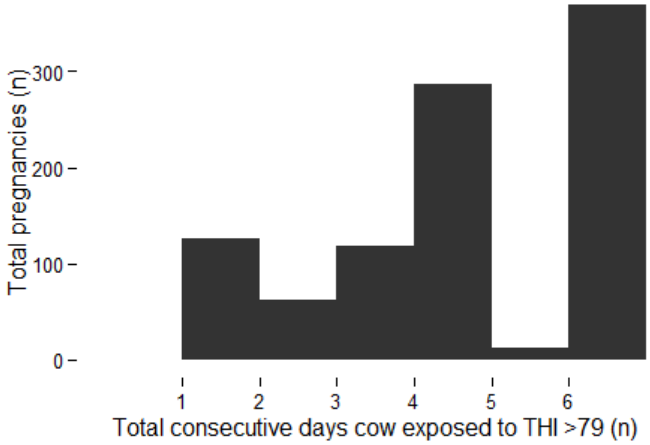


Figure 4. Distribution of pregnancies for total number of days where temperature-humidity index at maximum daily temperature exceeded 79 for consecutive days. n=977.

The numbers of calves born by research site, breed and dam year of birth are presented (Table 2).

Table 2. Number of calves born by breed, dam calving season of birth, by research site

	Brian Pastures		Spyglass		
Dam cohort[a]	Brahman	Composite	Brahman	Composite	Totals
2001	9	18	4	0	31
2002	14	42	3	1	60
2003	6	19	1	0	26
2004	0	4	0	2	6
2005	0	8	0	4	12
2006	3	15	0	6	24
2007	8	25	0	0	33
2008	19	16	6	4	45
2009	28	22	9	5	64
2010	67	28	22	4	121
2011	81	66	20	34	201
2012	16	52	10	22	100
2013	29	78	55	79	241
Totals	280	393	130	161	964

[a]The year of branding and weaning group of the dams

The majority of calves at both sites were born in November with calving tending to start earlier in the year at Brian Pastures (Figure 5). Neonatal mortality rates at Brian Pastures and Spyglass were similar: 3.1% (683 live births) and 4.7% (297 live births), respectively (p=0.2). Neonatal calves counted as mortalities included those that were removed from the cow and fed artificial milk (2 at Spyglass and 1 at Brian Pastures) and those euthanased (4 at Spyglass). Neonatal calves that were artificially reared or euthanased were recorded to have experienced one of the following: death or absence of dam (n=3 at Spyglass; n=1 at Brian Pastures), calf unable to stand or suckle (n=2), or an old dam that apparently was not producing enough milk (n=1). At each site, the incidence of neonatal mortality did not exceed 5% within any month of calving. At both sites, the percentage of live calves born with low vigour (vigour score <3) did not exceed 3% within any month. The design effect for clustering for being born at either Brian Pastures or Spyglass was 4.4.

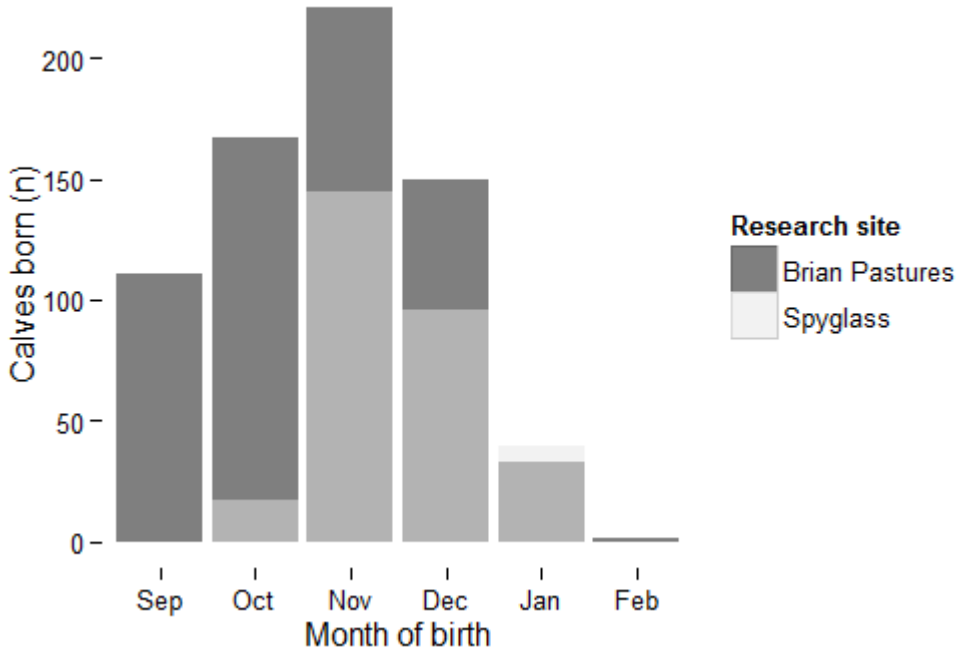


Figure 5. Frequency of calves born at Brian Pastures and Spyglass within each calendar month between the 11/September/2015 and 01/February/2016

Brahman calves had a higher percentage within the birth weight<28 kg category than composite calves (Figure 6). Calves of dams that were at Spyglass throughout pregnancy had a higher percentage of calves born at <28 kg than calves of cows that were at Brian Pastures throughout pregnancy (Figure 6). Cows that were moved from Spyglass to Brian Pastures at approximately day 209 of pregnancy had a similar percentage of calves with low birth weights to cows that were at Brian Pastures throughout pregnancy (Figure 6).

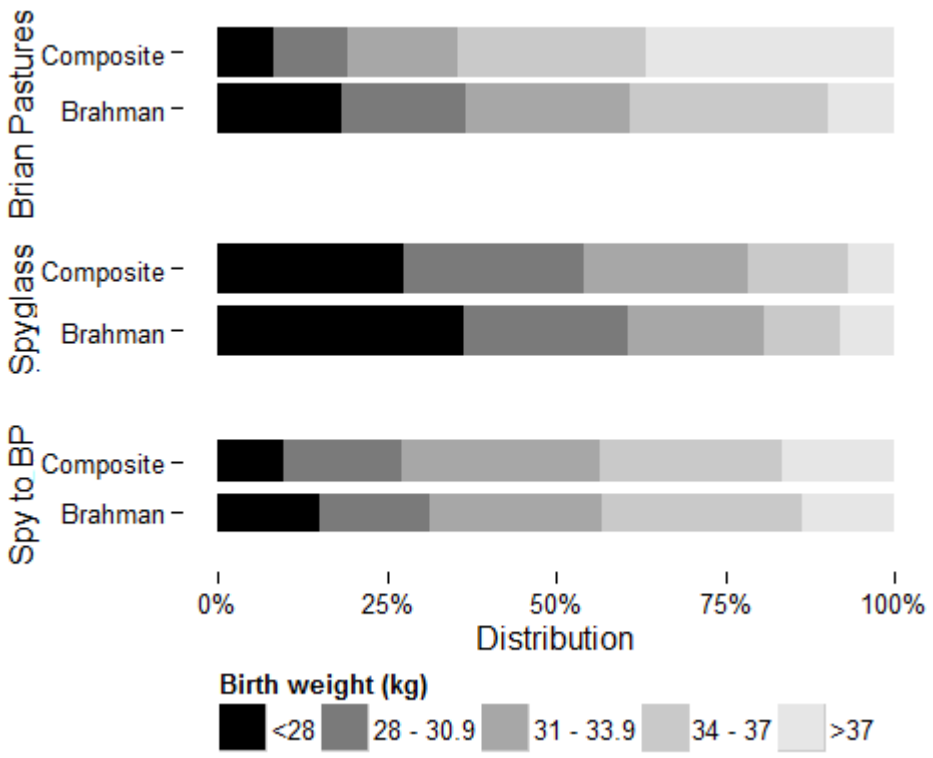


Figure 6. Percentage distribution of calf birth weights by breed, within research site. Birth weight categories are based on 20 percentile intervals. Brian Pastures n= 389; Spyglass n=297; Spy to BP= cows were moved from Spyglass to Brian Pastures during mid- to late-pregnancy, n=294.

The observed percentage of calves exposed to risk factors did not exceed eight percent for six of the variables studied (Table 3). Calves with vigour scores less than three were, on average, 60 times (IRR 60 95% CI 29-122.64, p<0.001) more likely to die compared with calves with vigour scores of three or greater. Compared with calving difficulty scores of one or less, calving difficulty greater the one inreased the incidence risk of neonatal mortality by a factor of 30 (IRR 30 95% CI 15 – 61; p<0.001). The frequency of dam body condition ≤2 was only eight percent and there appeared to be a non-significant trend for increased risk of neonatal mortality for calves of these cows (IRR 1.3 95% CI 0.64-2.64, p=0.67; Table 3). For the variables studied where the incidence of exposure was greater than eight percent, all risk ratios for neonatal mortality were non-significant, except for calf birth weight (IRR 3.5 95% CI 1.73-7.11, p<0.001 Table 3). Research site and period of birth had no significant effect on neonatal mortality (Table 3).

Table 3. The frequencies at which calves were exposed to high risk for mortality and crude risk ratios for impact of risk factors on neonatal mortality, for Spyglass and Brian Pastures research stations from September 2015 to February 2016.

Variable	Neonatal mortality n	Total born, n (%)	Percentage incidence of neonatal mortality (95% CI)	Risk ratio (95% CI)	p-value
Research Site					0.21
Brian Pastures	21	683 (70%)	3.08% (1.91% – 4.66%)	REF	
Spyglass	14	297 (30%)	4.71% (2.60% – 7.78%)	1.53 (0.76 – 3.11)	
Period of birth					0.52
Early	22	565 (58%)	3.89% (2.46% - 5.84%)	REF	
Late	13	415 (42%)	3.13% (1.68% - 5.3%)	0.8	
Total days THI >79 in early pregnancy					0.82
<15	1	23 (2%)	4.35% (2.39% - 4.82%)	REF	
≥15	33	954 (98%)	3.46% (2.39% - 4.82%)	0.80 (0.39 - 1.61)	
Total days THI >79 in late pregnancy					0.65
<15	19	509 (52%)	3.73% (2.26% - 5.77%)	REF	
≥15	15	468 (48%)	3.21% (1.8% - 5.23%)	0.86 (0.42 - 1.74)	
Cow Parity					
Multiparous	20	726 (75%)	2.75% (1.69% - 4.22%)	REF	0.18
First lactation	11	244 (25%)	4.51% (2.27% - 7.92%)	1.64 (0.81 - 3.32)	
Dam body condition score					0.67
>2	25	880 (92%)	2.84% (1.85% - 4.17%)	REF	
≤2	3	81 (8%)	3.7% (0.77% - 10.44%)	1.3 (0.64-2.64)	
Udder size score					0.41
≤4	26	944 (99%)	2.75% (1.8%1 - 4.01%)	REF	
>4	1	14 (1%)	7.14% (0.18% - 33.87%)	2.59 (1.27 - 5.26)	
At least two teats of score 5					0.002
No	31	964 (98%)	3.22% (2.20% - 4.53%)	REF	
Yes	4	16 (2%)	25% (7.27% - 52.38%)	7.77 (3.83 - 15.77)	
Maternal protectiveness					0.46
≥3	21	910 (95%)	2.31% (1.43% - 3.51%)	REF	
<3	2	49 (5%)	4.08% (0.50% - 13.98%)	1.77 (0.87 - 3.59)	
Calf birth weight					<0.001
>28 kg	17	788 (82%)	2.16% (1.26% - 3.43%)	REF	
≤28 kg	13	172 (18%)	7.56% (4.09% - 12.58%)	3.5 (1.73 - 7.11)	
Calving difficulty score					<0.001
≤1	24	964 (99%)	2.49% (1.60% - 3.68%)	REF	
>1	6	8 (1%)	75% (34.91% - 96.82%)	30.13 (14.85 - 61.12)	
Calf vigour score					<0.001
≥3	12	945 (98%)	1.26% (0.65% - 2.19%)	REF	
<3	16	21 (2%)	76.19% (52.83 - 97.78)	60.44 (29.79 - 122.64)	

REF: Reference value. 95% CI = 95% Confidence interval

For cows at a single site throughout both pregnancy and calving, the frequency at which calves were exposed to high risk was greater at Spyglass than Brian Pastures for: number of days at which temperature-humidity index>79, first lactation dams, and birth weight <28 kg (Figure 7). Cows moved from Spyglass to Brian Pastures at around late gestation were exposed to a high temperature-humidity index for a total number of days that was intermediate to those cows that remained at Spyglass and those that remained at Brian Pastures throughout pregnancy (Figure 7). Cows moved from Spyglass to Brian Pastures did not include first lactation cows. Cows moved from Spyglass to Brian Pastures had relative frequencies of poor maternal protectiveness and low birth weight that were lower than the cows that remained at Spyglass, and were similar to cows originating at Brian Pastures. For other variables, the distribution of calves exposed to high risk was similar across sites (Figure 7).

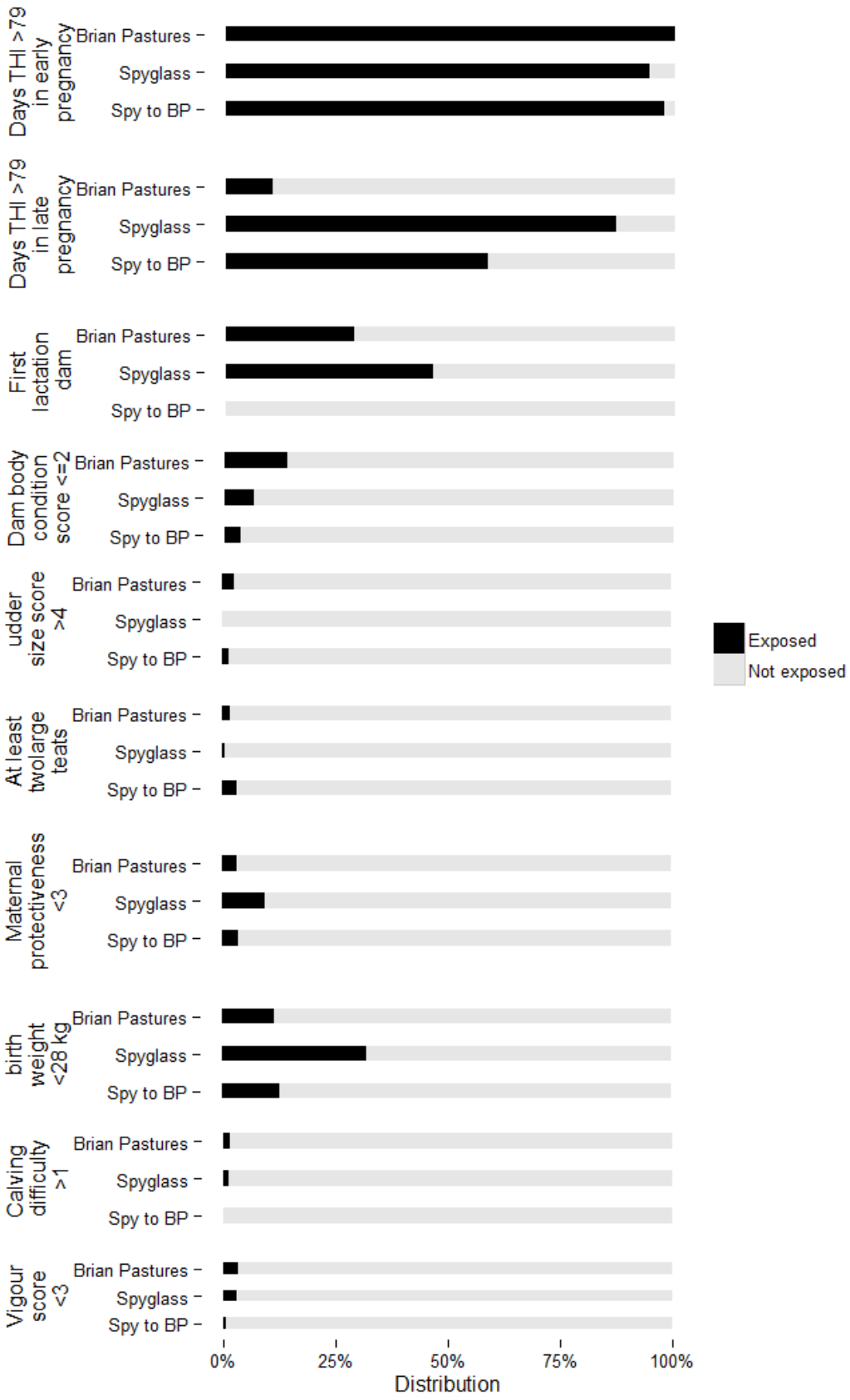


Figure 7. Frequency at which calves were exposed to risk factors by research site. Brian Pastures n= 389; Spyglass n=297; Spy to BP= cows were moved from Spyglass to Brian Pastures during mid- to late-pregnancy, n=294.

Of calves dying in the neonatal period, 60% and 40% were exposed to two or fewer and more than two risk factors, respectively (Table 4). The main clustering of calves with low vigour and with dams of with low maternal protectiveness were also mostly aligned with either low dam body condition score or a dam in their first lactation. Some of the cluster of low birth weight and low calf vigour also appeared to be aligned with calving difficulty within first lactation cows. Approximately half of the calves within this main cluster were also of low birth weight (Table 4). Ten calves that died were only exposed to high risk for low birth weight, low vigour, or both low birth weight and low vigour (Table 4). Variables for which the highest proportion of neonatal calves were exposed to high risk were poor calf vigour (n=22) and low calf birth weight (17; Table 4). Within neonatal mortalities, the risk variables in multiparous cows appeared clustered with poor body condition to a greater extent than for first lactation cows (Table 4).

Table 4. Neonatal calf mortalities and the high risk factors for which they were exposed to at Spyglass and Brian Pastures research stations from September 2015 to February 2016. Shading with an 'X' shows that calves were exposed to the high risk as described in column heading.

Identification number of dead calves	First lactation	Dam body condition score ≤2	Calf birth weight ≤28	Calf vigour score ≤2	Maternal protectiveness ≤2	Calving difficulty >1	Udder size 5	At least two teats of score five
16B107	X	X	X	X	X			
16B186		X	X	X	X	X		
16B187		X	X	X	X	X		
16B001		X	X	X	X			
16S035		X	X	X	X			
16B175		X	X	X	X			
16B011				X	X	X		
16B050	X			X	X	X		
16S169	X			X	X	X		
16B164	X			X	X			
16S099	X			X	X			
16S106		X		X	X			
16X406		X		X	X			
16S068		X		X	X			
16S195	X	X	X					
16B325	X			X				
16S231	X			X				
16B065	X		X					
16B344			X					X
16X489			X					X
16B214			X	X				
16B289			X	X				
16S123			X	X				
16B386			X	X				
16S295	X					X		
16X347		X						
16S174		X						
16X404								X
16X585								X
16S043				X				
16S237				X				
16S018			X					
16S074			X					
16X510			X					
16X402								

4.4 Discussion

The environment of the northern forest is associated with higher foetal and calf mortality than the southern forest (McGowan *et al.*, 2014). In contrast, this study demonstrated how a low proportion of calves exposed to risk factors in any situation results in low calf mortality. McGowan *et al.* (2014) reported that of all country types in northern Australia, the harsh environment of the northern forest had the highest incidence of foetal and calf mortality. The incidence of neonatal mortality at Spyglass of five percent is lower than the nine percent incidence of foetal and calf mortality that was reported by (McGowan *et al.*, 2014). Nine percent was reported to be the achievable incidence of foetal and calf mortality in the northern forest (McGowan *et al.*, 2014), where Spyglass is situated. The neonatal mortality incidence of three percent at Brian Pastures reflected the achievable incidence of foetal and calf mortality (two percent) in the southern forest where Brian Pastures is situated (McGowan *et al.*, 2014). It is not surprising that the incidences of neonatal mortality in this study are well aligned with the incidence of foetal and calf mortality previously reported (McGowan *et al.*, 2014), given that the majority of foetal and calf loss occurs during neonatal life (Bunter *et al.*, 2013).

A low frequency of cows in poor body condition (eight percent) and low incidence of neonatal calf mortality is consistent with McGowan *et al.* (2014)'s finding that poor dam body condition is associated with increased risk for foetal and calf mortality, within cows at risk of low phosphorus status. The trend of increased neonatal mortality in poor cows compared to those in better condition was not significant, although this may be due to the low frequency of poor cows. This speculation is supported by the fact that in McGowan *et al.* (2014)'s study, the frequency of poor cow body condition in the northern forest was 25%. Additionally, Vargas *et al.* (1999)reported that within Brahman cows of third or greater parity, calf survival from birth to weaning was 88.8% ± 10.76% and 94.1% ± 4.74%, respectively for cows in "thin or worse condition" or with some fat cover. The frequency of cows in "thin or worse condition" and with some fat cover was 6% and 29%, respectively. Therefore the trend of increased calf mortality in poor-condition cows may be non-significant in both Vargas *et al.* (1999) and the present study due to low frequencies of cows in poor condition. The positive association between incidence of foetal and calf mortality and poor cow condition as reported by McGowan *et al.* (2014) did not account for cow mortality. The actual strength of association between poor cow condition and foetal and calf mortality may be higher than the report, given the high risk of cow mortality for those in poor condition (McGowan *et al.*, 2014, Fordyce *et al.*, 1990, Mayer *et al.*, 2012)and that all mortalities of lactating cows in Fordyce *et al.* (1990)were associated with calf mortality.

In the present study the majority of neonatal calves were observed to be well hydrated and strong. Given the reported positive associations between cow nutrition and milk yields (Castells *et*

al., 2014, Bell *et al.*, 2000, Cowan *et al.*, 1980, Coulon and Remon, 1991), between cow body condition and calf survival (McGowan *et al.*, 2014, Vargas *et al.*, 1999), a high relative frequency of cows in poor body condition may result in reduced milk yields and increased relative frequency of calves experiencing dehydration mediated neonatal mortality. However, good nutritional management resulted in the low frequency of cows in poor body condition and limited the opportunity for dehydration-mediated neonatal mortality.

The low frequency of cows in poor body condition, the low frequency of low birth-weight calves, and the low frequency of weak newborns calves in this study is consistent with studies demonstrating the positive association dam nutrition during gestation with calf vigour (Bull *et al.*, 1974) and birth weight (Fordyce *et al.*, 1993, Micke *et al.*, 2010, Sullivan *et al.*, 2009a). The birth weights of calves from cows moved from Spyglass to Brian Pastures in late pregnancy appeared to reflect that of the cows present at Brian Pastures throughout pregnancy. The frequency of low birth weight calves from cows which remained at Spyglass was much higher. The lower frequency of low birth weight calves at Brian Pastures compared to Spyglass may at least partially explain the trend for lower incidence of neonatal mortality at Brian Pastures. Low birth weight may at least partially explain low calf vigour. This observation is consistent with that of Schmidek *et al.* (2013) who reported that tropically-adapted calves of low birth weight were at higher risk of poor birth vigour.

The low incidence of weak newborns and dystocia in this study is consistent with Riley *et al.* (2004) who documented an association of dystocia with reduced calf vigour in Brahman cattle. Holroyd (1987) previously reported a trend of decreasing incidence of dystocia in cows of increasing *Bos indicus* content. The low incidence of dystocia in the present study is consistent with Wythe (1970), where frequency of dystocia was low in Brahmans.

In Friesian calves bottle-fed their first colostrum *ad*-libitum, uptake volume was positively associated with calf vigour as defined by attempting to stand within the first hour of life (Vasseur *et al.*, 2009). Kim et al (1988) reported that Brahman calves without an instinct or ability to suckle took 409 minutes from birth until suckling, while other Brahman calves took 227 minutes. A delay in milk consumption for 2 to 3 days may lead to mortality in tropically adapted calves (Fordyce *et al.*, 2015). Therefore, low calf vigour and low ability to seek teats and access milk may be an ultimate reason for dehydration mediated mortality.

This study demonstrated that intermittent periods of heat stress around calving did not lead to higher neonatal calf mortality. This does not disqualify the hypothesis that heat stress impacts are mediated by direct impacts on cows and calves as the temperature-humidity index effect reported by McGowan et al. (2014) was associated with at least 15 consecutive days of temperature-humidity index >79 in the month of birth. Heat stress may reduce blood flow to the foetus

(Reynolds *et al.*, 1985), which may in turn reduce vigour of newborns. Alternatively, heat stress may reduce mammary development (Tao and Dahl, 2013) and milk yields (Collier *et al.*, 1981, West *et al.*, 2003) as in dairy cows. In Brahman cows, there is an apparently-linear decline in milk yield as ambient temperature increases from 35^{o}C to 43^{o}C (Brody, 1956). This increase in temperature appeared to explain a drop in milk production of approximately 1 kg/day.

For the effect of heat stress on reducing milk yields in dairy cows, only 35% (Rhoads *et al.*, 2009) to 50% (Wheelock *et al.*, 2010) was explained by the associated reduction in dietary intake. The balance of the effect is likely explained by changes in post-absorptive metabolism. The main difference between dairy cows that are heat stressed, and in thermoneutral conditions though in a similar energetic state to their heat stressed counterparts, is that the heat stressed cows have an inability to mobilise and use non-glucose fuels of lactation, including NEFAs and ketones (Baumgard and Rhoads, 2012). The above effects of heat stress are driven by a reduction in lactose secretion (Rhoads et al., 2009, Wheelock et al., 2009). The secretion of lactose and monovalent ions by mammary tissue is a primary driver of milk volume secreted from mammary tissue (Holt, 1983). Cowley (2013) reported that heat stress was associated with change in percentage casein of milk beyond what occurs due to reduction in dietary intake. Such a minimal change in milk composition is likely to be of limited importance to calf hydration when compared to the effect of heat stress on reduced milk yield. The effects of heat stress or dry matter intake have a 2-day lag effect on daily milk yield (compared to days 1 or 3 prior), with linear relationships between heat stress, dry matter intake and milk production (West *et al.*, 2003).

In dairy cows, there was some decrease in milk yields associated with heat stress during the preceding dry period (Tao *et al.*, 2011), possibly driven by a reduced conversion efficiency of nutrients to body reserves during the dry period.

Reduced milk availability of neonates has direct impact on fluid balance especially during periods of high heat load. A study in Brahmans by Wythe (1970) showed that relative frequency of low birth vigour varied from 3.5% to 11.8% over a number of years, and that birth vigour was impacted significantly by sequence of birth in the calving season, temperature at birth and precipitation at birth. Edwards (1982) reported that time of birth in the calving season may at least partially explain vigour to stand and suckle, an apparent function of ambient temperature. Further investigation is required to determine whether these heat stress directly affect animals, or other possible other impacts occur, which may include higher rainfall after a dry spell and therefore boggy conditions for calves or reduced efficiency of pasture growth (Bastin *et al.*, 2014, Hall *et al.*, 1998).

In this study temperature-humidity index had peaks of similar magnitude that occurred in early gestation in both northern forest and southern forest and around calving in the northern forest.

The association of increased foetal and calf mortality with prolonged heat stress around calving has been reported to occur in the southern forest and not the northern forest (McGowan *et al.*, 2014). The lack of association in the northern forest may be explained by a limited variation in heat stress around calving. This speculation is further supported by a lower frequency of days of high heat stress days around calving in the southern forest than in the northern forest, and lower frequency of high heat stress days around calving in the southern forest than during early pregnancy in either region.

Animal level risk factors for calf mortality including large teats and large udders had very low frequencies. These risk factors pose difficulty for the calf in accessing milk (Sapp *et al.*, 2004, Frisch, 1982, Ventorp and Michanek, 1992, Bunter *et al.*, 2013). Udder and teat size are under significant genetic control (Bunter and Johnston, 2014). The very low frequency of large udder and large teats allowed opportunity for expression of any possible effects of dam nutritional deficiency or environmental stress on neonatal mortality. Large teats do not pose an issue for 3-4 year old cows (i.e. around the age of first parity) though increase in relative frequency of large teats with age (Frisch, 1982). With the low frequency of large teats, a non-significant tendency was expressed where there was a higher incidence of neonatal mortality in first lactation dams compared to multiparous dams. The non-significance may be explained by a low frequency of first lactation dams. This speculation is supported by a higher median incidence of foetal and calf mortality for cows rearing their calf in their first lactation (11.1%) than those at later lactations (8.1%;McGowan *et al.*, 2014). A higher risk for calf mortality in first lactation dams may be at least partially explained by the additional nutritional strain of growing. This speculation is supported by Vargas *et al.* (1999), where within first lactation dams in good body condition, large and medium frame cows had respective calf survival rates of 47.9±11% and 85.2±4.71%. This may also explain the nutritional strain in larger frame cattle causing higher maintenance requirement (Green *et al.*, 1991) in addition to growth requirement in the first lactation females.

4.5 Conclusion

Low incidence of calf mortality may be achieved in any country type, provided a low proportion of calves born are exposed to risk factors. For many breeding herds, a high proportion of calves are exposed to risk factors for calf mortality. Therefore, to reduce the incidence of calf mortality, risk factors targeted for reduction should be those that are not only associated with calf mortality but also those that exert impact of a high proportion of calves born.

Acknowledgement

Nicholas Brazier and Joanne Campbell are gratefully acknowledged for their assistance in recording risk factor data in the field.

5. An interactive model to predict milk deprivation mediated calf mortality in northern Australia

5.0 Abstract

A simple predictive model was constructed to understand the relationship between milk delivery and hydration and therefore predict if neonatal calves will experience sufficient dehydration to result in mortality as a result of various milk delivery regimes. Model calves were set to be born at either maximum or minimum daily temperature. Model calves were also compared for long term minimum and maximum temperature averages at Charters Towers, Cloncurry and Brian Pastures, Queensland. Although some model components were based on that of *Bos indicus* calves (e.g. rate of dehydration when milk deprived), other information was not available and was therefore used from appropriate sources (e.g., increase in live weight per unit milk delivery data was used from *Bos taurus* calves). Calf body solids and body fluids were treated as separate compartments in the model. This model demonstrated that a difference in milk uptake volume as little as one litre per 12 hour period may mean the difference between neonatal survival and mortality. This result emphasises how any risk factor to milk delivery may impact neonatal calf mortality. Experimental data should be collected on *Bos indicus* calves to confirm model predictions.

5.1 Introduction

Across mammalian species, the most critical stage of life is the neonatal period (within a week of birth), a time at which they are at high risk of inadequate milk consumption (Nowak *et al.*, 2000). Tropically adapted beef breeding herds are no exception, as during the period from positive pregnancy diagnosis to weaning, 67% (Bunter *et al.*, 2013) and 43.8%[5](Holroyd, 1987) of mortality occurred during neonatal life. Despite the risk of dehydration mediated mortality (Fordyce *et al.*, 2015), no study has investigated a possible association between milk deprivation and neonatal mortality in northern Australian breeding herds. Increased foetal and calf mortality is associated with poor dam nutrition during months prior to parturition and high heat stress around parturition (McGowan *et al.*, 2014). Cow milk production and its associations with nutrition (Coulon and Remon, 1991, Castells *et al.*, 2014, McBryde *et al.*, 2013) and heat stress (Tao *et al.*, 2011, Collier *et al.*, 1981, Brody, 1956) are well established. Low calf vigour has been associated with poor dam nutrition during gestation (Bull *et al.*, 1974) and low vigour has been associated with delayed

[5] Although neonatal calf mortality rates were not reported in Holroyd (1987), counts were reported for number of pregnancies studied (3285), total losses between pregnancy diagnosis and weaning (516), calf mortality within 48 hours of life (170) and calf mortality between hour 48 and day 7 of life (56). Therefore neonatal mortality rate was calculated as percentage of total foetal and calf mortalities that occur during the first week of life. Holroyd also reported a pre-natal loss of 3.5% of pregnancies.

suckling behaviour (Vasseur *et al.*, 2009) and mortality (Muller 2007). In addition, Fordyce *et al.* (1990) reported that in Brahman crossbred cows, those that were lactating had highest risk of mortality and that all mortalities of lactating cows were associated with calf mortality. Therefore, the risk factors for foetal and calf mortality, as reported by McGowan *et al.* (2014) may be mediated by the above risk factors.

A simple predictive model was constructed to understand the relationship between milk delivery and hydration and therefore predict the magnitude of dehydration in neonatal calves. The main general hypothesis was that under certain conditions of low/delayed milk consumption or high ambient temperature, calves suffer dehydration mediated mortality during neonatal life.

5.2 Model construction

Haigh *et al.* (1920), Dalton (1964) and Fordyce *et al.* (2015) reported that fully-hydrated calves are approximately 74% body water; therefore, this value is used for euhydrated calves in the model. Yates *et al.* (1970) reported that for nine Holstein-Hereford crossbred calves, body water as a percentage of live weight only changed from 80% to 78% between birth and the second week of life. Given this small change, calves were assumed to be 74% body water at euhydration, throughout neonatal life.

Maintaining hydration is a dynamic process where the body continuously dehydrates and fluid is replenished at varying volumes and intervals of time (Greenleaf, 1992). Fordyce *et al.* (2015) reported that non-suckling neonatal calves lose live weight at rates of 0.18%/hr at night and 1.1%/hr at an ambient temperature of 39^{o}C. These reported losses include that accruing through urination, faeces and insensible losses (i.e., breathing, cutaneous evaporation and perspiration). Brody (1956) and Finch (1973a) reported how adult *Bos indicus* cattle only use evaporative means of thermoregulation at ambient temperatures exceeding 27^{o}C. In this model, rate of dehydration was assumed to be 0.18%/h at ≤27^{o}C and to linearly increase to 1.1%/h between ambient temperatures of 27^{o}C and 39^{o}C. Figure M1 visualises body water loss and other model components.

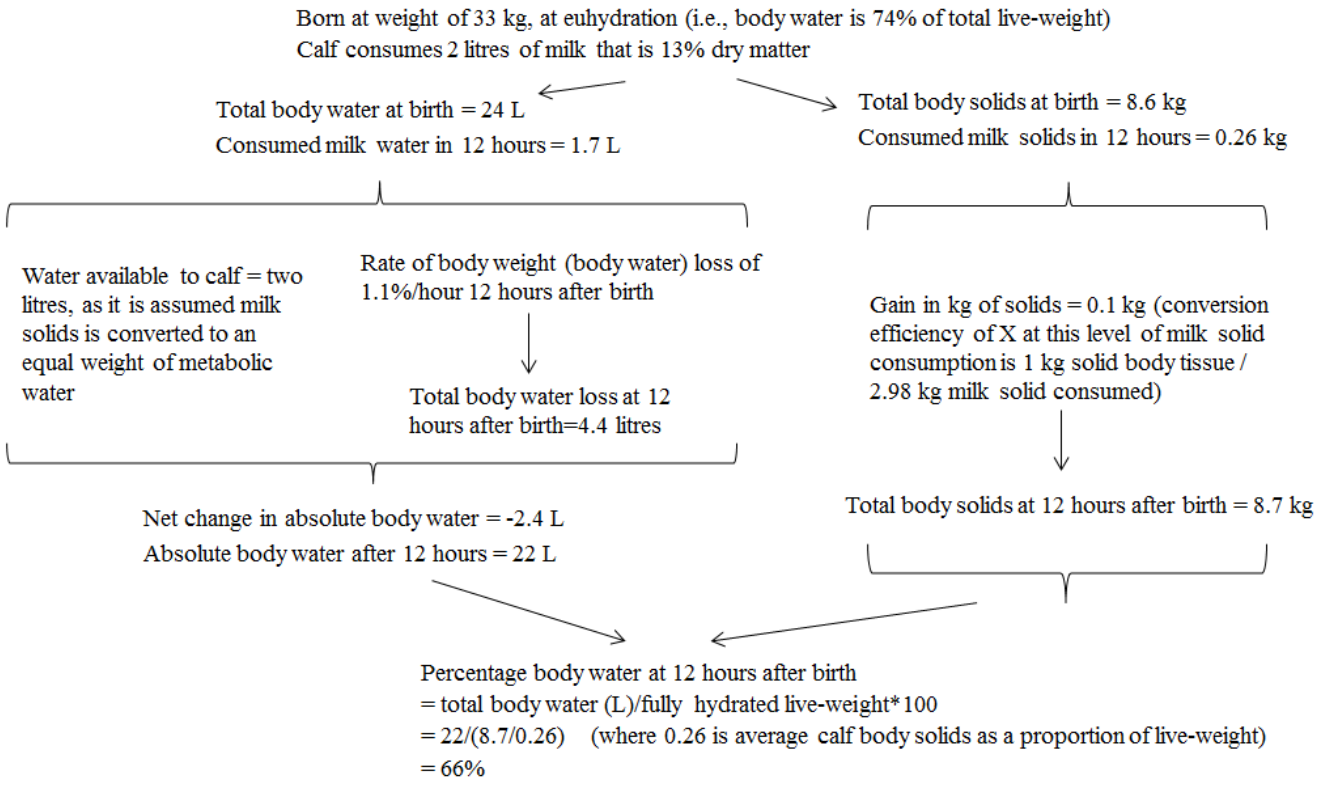


Figure M1. Flow diagram for visualisation of model components. Imputed data for calf 1A (born at minimum ambient temperature) for the first 12 hours of life are used as an example.

Fordyce *et al.* (2015) reported that Brahman-crossbred calves may lose the ability to suckle if they lose in excess of 15% body weight, or decrease in body water by 20% from euhydration. Therefore, for the model, dehydration-mediated mortality was assumed to occur for calves that reach ≤ 59% (=(1-0.2)*0.74) body water.

In hyperthermic acutely dehydrated rabbits, the decrease in body weight of 5% due to dehydration was proportional to the decrease in plasma volume and total body water (Reese and Haines, 1978). In acutely dehydrated rats, the percentage decrease in body weight was similar to the percentage decrease in body water (Horowitz and Borut, 1970). In sheep that were water-deprived for 120 hours, live weight decreased by 23% and plasma volume, a measure of hydration, by 45% (Macfarlane *et al.*, 1956). In a non-obese man, fasting and only consuming water for 42 days lead to a weight loss of 25% (Jackson *et al.*, 2006) which equates to 0.6% loss in solid tissue mass per day. The above studies indicate that weight loss experienced during acute dehydration is primarily through loss of body water, with very little loss of tissue through catabolism. It is therefore assumed in acute dehydration of neonatal calves, weight loss is practically accounted for by water loss.

Detailed studies on initiation of lactation are limited in *Bos indicus*, though in dairy cows there was minimal milk secretion during the 4 days prior to parturition and an approximate doubling to tripling of milk yield between parturition and day 3 postpartum (Hartmann, 1973, Wheelock *et*

al., 1965). Kim et al (1988) reported that Brahman calves without an instinct or ability to suckle at birth took an average of 409 minutes (6.8 hours) from birth until suckling, while other Brahman calves took an average of 227 minutes (3.8 hours). In Friesian calves bottle-fed their first colostrum *ad*-libitum, uptake volume was associated with calf vigour, as defined by attempting to stand within the first hour of life (Vasseur *et al.*, 2009). Therefore, daily volume of milk uptake of neonatal calves, whether limited by cow lactation yield or calf ability to suckle, is likely to be increasing throughout neonatal life. Milk consumption patterns in the model were based on increasing or consistent volumes of milk uptake through neonatal life.

Literature is very limited for calves with hypernatraemic dehydration. In humans that were dehydrated to change in plasma volume by -7.5%, with oral rehydration, a 10% increase in plasma volume was detected at 15 minutes post-rehydration (Godek, 2000). The 15 minute measurement was the first measurement for detection of rehydration and there was no further increase in plasma volume at 30 or 45 minutes post-rehydration (Godek, 2000). Therefore, in dehydrated individuals, it is likely that rehydration is a rapid process that occurs before 15 minutes. Therefore a similar rate of rehydration may occur in dehydrated neonatal calves, given that they are pre-ruminants and use similar digestive processes to monogastrics (Radostits and Bell, 1970), with similar regulation of abomasal emptying (Sen *et al.*, 2006) into the intestines where the majority of milk water is absorbed (Smith, 1961). Additionally, in calves, the majority of water of ingested milk rapidly passes into the intestines after feeding (Mylrea., 1966), while much milk protein and fat clots in the abomasum (Logenbach and Heinrichs, 1998)to slowly pass into the intestines (Petit, 1987).In addition, urea, which is soluble in water when injected into the bloodstream, will equilibrate through tissues within an hour (Dalton, 1964). The above studies support the assumption of rapid (likely instantaneous) absorption of water from ingested milk, especially in dehydrated calves.

The above studies also support the assumption that in dehydrated calves, efficiency of converting ingested milk water to body water is 100%, until full hydration is reached. The total water available for the calf was assumed to be equal to the volume of milk consumed, given that milk solids have been reported to produce an amount of metabolic water similar to the weight of milk solids (Macfarlane *et al.*, 1969). Any increases in fluid accretion exceeding 74% of total body weight are assumed to be excreted.

Neonatal calf growth is determined by solid and fluid accruing as tissue growth and is primarily a function of milk intake (Totusek *et al.*, 1973, Black, 1982, Castells *et al.*, 2014, Bartle *et al.*, 1982, Montsma, 1960).Mendonça *et al.* (2002) reported that in a herd of first lactation Hereford cows, *Bos indicus* crossbred and *Bos taurus-Bos taurus* crossbred calves did not differ in growth. It is likely that the Hereford cow groups did not differ overall in milk production and therefore indicating that *Bos indicus* and *Bos taurus* calves may not differ greatly in efficiency of converting

milk to live weight. Reynolds *et al.* (1978) reported that coefficients of correlation between calf growth for the first 83 days of life and milk yield as measured on day 83 were similar for Brahman, Brangus, Angus and Afric-Angus (0.42, 0.53, 0.54 and 0.52, respectively). The above studies support the assumption that milk uptake is the most important driver of calf growth and not calf genotype. Given very limited data on the relationship between milk uptake and growth in tropically-adapted neonatal calves, it is assumed that conversion efficiency of milk solids to tissue is similar to that of Friesian calves, for which data is available.

Khouri and Pickering (1968) reported that for Friesian calves fed varying quantities of a milk replacer, total intake of milk dry matter during the first two weeks of life ranged from 4.7 kg to *ad libitum* intake of 7.41 kg. Respective efficiencies of conversion of milk replacer to live weight (kg milk powder/kg live weight gain) ranged from 2.98 and 1.19. Holmes *et al.* (1968) reported Brahman-crossbred milk to be 4.2% and 8.9% milk fat and non-fat solids. Therefore milk was assumed to be 13% dry matter.

For each 12-hour period of neonatal life, model calves were re-calibrated for percentage hydration, calculated as body water (L)/[body water (L) + body solids (kg)]. Recalibration is at 5 AM and 5 PM, which coincide approximately with the maximum and minimum daily temperatures, respectively.

Using the above information, an interactive model was constructed in Microsoft Excel version 14.0.7177.5000. As the output variable, percentage body water, was only calculated for 12 hourly intervals, a smoothing function used to show overall trend of hydration status. The smoothed lines were plotted using the ggplot2 package (Wickham, 2009) within R (R Core Team, 2014).

5.3 Model inputs and outputs

The main model outcome variable is total body water (L) as a percentage of live weight (kg), which is based on estimations calf total body water and total body solids.

The primary input variables were birth weight, milk uptake and ambient temperature. Bunter and Johnston (2014) reported an average Brahman birth weight of 32.7±5.08. Birth weight for calves was therefore set at 33 kg. Milk uptake pattern throughout neonatal life was determined by three imputed values of milk uptake (L/12 hours), for the periods starting at 0, 2.5 and 5 days from birth. Milk uptake during each of the other 12 hour periods was setup to change linearly between imputed values. Ambient temperatures were set to switch from daily every 12 hours, to simulate maximum and minimum temperatures occurring at approximately 5:00 AM and 5:00 PM.

Two scenarios were run in the model. In scenario one, various patterns of milk consumption were compared for percentage body water through neonatal life. Imputed maximum and minimum daily temperatures were 27^{o}C and 39^{o}C, respectively. Within each milk consumption pattern occurring throughout neonatal life, comparison was made between calves that were born during maximum daily temperature and those born at minimum daily temperature. In scenario two, two milk consumption patterns were investigated. Within each milk consumption pattern, ambient temperatures imputed were varied to reflect long term averages at the sites of Charters Towers, Cloncurry and Gayndah in Queensland. The maximum and minimum temperatures used for each location are below (Table 1). All calves in scenario two were born at maximum daily temperature.

Table 1. Long-term average for maximum and minimum temperatures in the month of November

	Charters Towers	Cloncurry	Gayndah
Maximum daily temperature	34.1	38	30.8
Minimum daily temperature	19.9	23.3	17.5

Temperatures are based on averages at the Charters Towers post-office recorded during 1983-1992, at Cloncurry airport during 1978-2008 and Brian Pastures during 1968-2008. From searching for these locations at the website: http://www.bom.gov.au/climate/data/index.shtml(Bureau of Meteorology, 2016a)

5.4 Results

In Figure 1, calves within one graph have the same pattern of milk uptake from birth to day seven of life. Therefore, a difference in the two lines within a graph simulates the difference in percentage body water for calves being born at maximum daily temperature or minimum daily ambient temperature. For example, in Figure 1B, the model calf being born at maximum temperature experiences decreasing ambient temperatures for the first 12 hours of life compared with the calf born at minimum ambient temperature that experiences increasing ambient temperature for the first 12 hours of life. In this case higher heat stress in early life predicted mortality in model one calf while the other model calf survived (Figure 1B). In all scenario one calves, being born at minimum ambient temperature lead to a reduction in body water throughout neonatal life, compared to being born at maximum ambient temperature (Figure 1). Comparing between calves within each Figure 1 graph, the opposite trends of minor peaks and troughs clearly reflect the impact of ambient temperature on rate of dehydration and the overall trends of

percentage body water through neonatal life are therefore primarily driven by volume of milk uptake.

When milk consumption patterns 1A and 1B are compared (for calves born at maximum ambient temperature, i.e., black lines) an increased milk uptake by one litre/12 hours at day 2.5 and 5 days from birth was associated with neonatal survival. When milk consumption patterns 1C and 1E are compared (for calves born at maximum ambient temperature), calf 1E had consumed 0.5 and 1 litre more milk at 2.5 and 5 days from birth, respectively. This additional milk consumption was associated with an extension of survival for an additional three days (Figure 1).

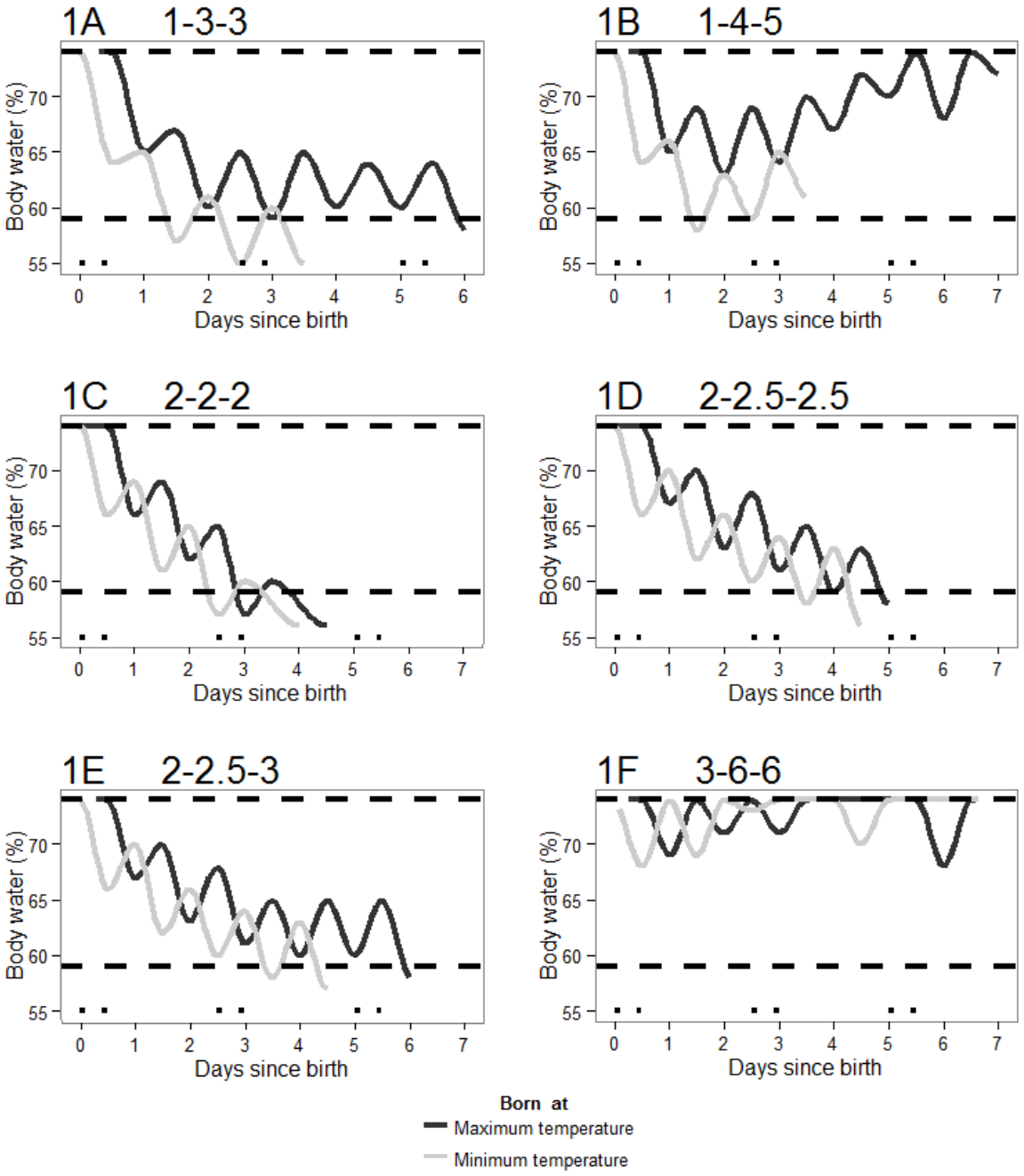


Figure 1 (Scenario 1). Calf body water as a percentage of live-weight, during neonatal life for calves born at maximum and minimum daily temperature. Graph headings are volume of milk consumed within the 12 hour periods starting from 0, 2.5 and 5days since birth, respectively, as shown by dots. Milk consumption increased linearly between these points. Upper broken line=full hydration, lower broken line=dehydration mediated mortality.

Figure 2 demonstrates how the long term averages for maximum and minimum temperature in different regions may impact on neonatal calf percentage body water. Overall rates of dehydration were highest at Cloncurry and lowest at Gayndah.

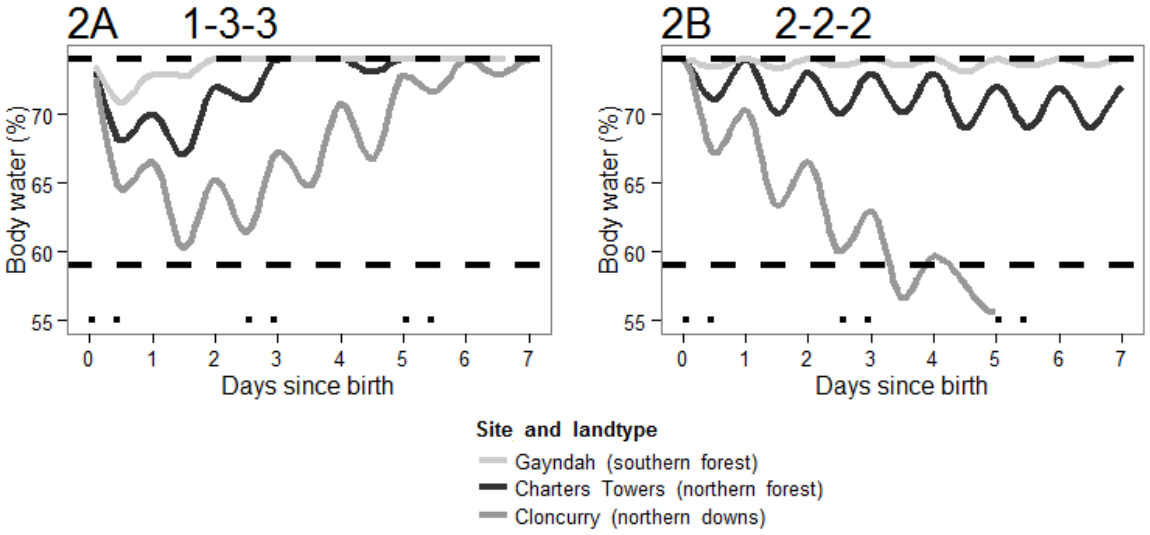


Figure 2 (Scenario 2). Calf body water as a percentage of live-weight, during neonatal life for different sites. Graph headings are volume of milk consumed within the 12 hour periods starting from 0, 2.5 and 5days since birth, respectively, as shown by dots. Milk consumption increased linearly between these points. Upper broken line=full hydration, lower broken line=dehydration mediated mortality.

5.5 Discussion

This model demonstrates that a difference in milk uptake volume as little as one litre per 12 hour period may mean the difference between neonatal survival and mortality. Reynolds *et al.* (1978) reported that that Brahmans produce 2.8 kg per 16 hours and McBryde *et al.* (2013) reported that Brahmans cows produced 3.3 kg per 24 hours. McBryde *et al.* (2013) also reported that a decrease in body condition score of one (on a one to five scale) was associated with a reduction in 24-hour milk yield of one kilogram. Therefore cow body condition may be important in driving calf milk uptake and neonatal survival or mortality. Other risk factors that limit milk delivery to calves, for example poor maternal instinct of poor calf ability to suckle, may also ultimately lead to dehydration mediated neonatal mortality.

Rates of dehydration were predicted to be lowest in the southern forest and highest in the northern downs. McGowan *et al.* (2014) reported that median foetal and calf loss was 5% and 8% in southern forest and northern downs respectively. McGowan *et al.* (2014) also reported that there foetal and calf loss was explained by prolonged heat stress around calving. Therefore, the impact of heat stress on neonatal rates of dehydration may at least partly explain differences in foetal and calf mortality between country types.

Although the general concepts demonstrated by this model are likely sound in principle, specific details should not be relied on. The assumption of dehydration is based on empirical data of eight milk deprived Brahman-crossbred calves (Fordyce *et al.*, 2015) that dehydrated to a point that results in mortality within 2-3 days. The concept of dehydration mediated mortality is likely sound, as Fordyce *et al.* (2015) reported that Brahman crossbred calves are likely experiencing dehydration mediated mortality if they lose approximately 15% of live weight due to milk deprivation. Horowitz and Borut (1970) reported that rats experienced dehydration mediated mortality at a similar weight loss of 14-22%. In Horowitz and Borut (1970)'s study, the period from the initiation of water deprivation to mortality was only eight to ten hours.

Horowitz and Borut (1970) reported that species differ in their ability to withstand dehydration mediated mortality, which may be explained by variation between species in the magnitude of dehydration that leads to their mortality, their ability to conserve total body water and their ability to adjust the proportions fluid in different body water compartments, to direct water to more vital compartments, for example, moving interstitial water to the intracellular water or plasma water compartments. Therefore, in reality, neonatal calves may vary in their ability to conserve body water and therefore their resistance to dehydration mediated mortality. This possible variation may be related to metabolic ability as determined by *in utero* development or genotype. Increased rates of cutaneous evaporation is the most effective means to increase heat dissipation when under increasing heat stress (Brody, 1956), though *Bos indicus-Bos taurus* crossbred calves (or *Bos indicus* composite breeds) likely need to lose more body water to thermoregulation under hot conditions that pure *Bos indicus* calves. This is supported by the finding of (Finch, 1985), where when ambient temperature exceeded skin temperature by 7^{o}C, evaporative heat loss as percentages of non-evaporative heat loss were ~190% and ~150% for Brahman crossbred and Brahman steers (Finch, 1985). The assumption of linear increase in rate of body water loss with increasing ambient temperature may approximate reality. This is supported by the results of Brody (1956), where water vaporisation rates in cattle increased approximately linearly for temperatures exceeding the minimum that leads to heat stress.

Calf growth is determined by solid and fluid accruing as tissue growth and is primarily a function of milk intake (Totusek *et al.*, 1973, Black, 1982, Castells *et al.*, 2014, Bartle *et al.*, 1982, Montsma, 1960). Therefore the assumption the efficiency of conversion of milk to live-weight is similar in *Bos taurus*(on which the model is based; Khouri and Pickering, 1968) is likely satisfied in principle. The calves of Khouri and Pickering (1968) were Friesians, and were studied for two weeks, and therefore the efficiencies of growth per milk solid consumption may not be accurate for neonatal tropically adapted calves. In addition, the Friesian calves were on average heavier than typical neonatal tropically-adapted calves, and absolute growth efficiencies were applied to the

model without adjustment for metabolic weight. The milk solids composition of Khouri and Pickering (1968)'s study may differ from milk of tropically-adapted beef cows.

The amount of metabolic water that is available to the calf from the solids consumed in milk is not clear. Macfarlane *et al.* (1969) reported that the amount of metabolic water produced is similar to the weight of milk solids, while Boulton (2015) explains that the metabolic water available to the calf may be negligible.

5.6 Conclusion

This model helps to understand how milk uptake volumes and ambient temperature (heat stress) impact on extent of neonatal dehydration. Therefore clarification is provided for the possible risk of dehydration mediated mortality across breeding herds of northern Australia.

6. General discussion

This thesis describes the first study of the variation in milk uptake for tropically-adapted neonatal beef calves in northern Australia. Clear evidence is provided that approximately half of a Brahman calf cohort received only maintenance levels of milk until day 3-4 of life and that the other calves were receiving volumes of milk that allowed them to grow rapidly from birth (Experiment 1). This variation in calf milk uptake may be due differences in cows' milk yield, or may alternatively be due to differences between calves in development and growth as a conceptus and subsequent ability to suckle, for which risk factors may be exerting their impact as early as peri-conception. Limited milk delivery under benign conditions in this research may explain high calf mortality rates where risk factors may be limiting either milk production or neonatal calf vigour.

In addition to the above experiment, risk factors for calf mortality were investigated in two extensively-managed situations where neonatal mortality rates were less than 5% when good nutritional management limited the opportunity for dehydration-mediated neonatal mortality (Experiment 2). Also, a simple mechanistic model was constructed to demonstrate the relationship between milk uptake pattern during the first week of life, i.e., neonatal life, and the occurrence of dehydration-mediated mortality. The above studies assist with investigating the importance of dehydration as a risk factor for neonatal calf mortality in northern Australia. Across mammalian species, the most critical stage of life is the neonatal period, a time at which they are at high risk of inadequate milk consumption(Nowak *et al.*, 2000). Tropically-adapted beef breeding herds are no exception, as during the period from positive pregnancy diagnosis to weaning, 67% (Bunter *et al.*, 2013) and 43.8%[6](Holroyd, 1987) of mortality occurred during neonatal life. There has been a gap

[6] Although neonatal calf mortality rates were not reported in Holroyd (1987), counts were reported for number of pregnancies studied (3285), total losses between pregnancy diagnosis and weaning (516), calf mortality within 48 hours of life (170) and calf mortality between hour 48 and day 7 of life (56). Therefore

in the knowledge on neonatal milk uptake in these herds. Possibly the first study focussed on dehydration-mediated mortality in milk-deprived calves was Fordyce *et al.* (2015). Fordyce *et al.* (2015) provided evidence that during milk deprivation, neonatal calves may experience dehydration mediated morality within 1-3 days.

Within Experiment 1, the delayed-growth calves reached the rate of growth that the other calves had from birth, but not until after day 3 of life. Calf growth is determined by solid and fluid accruing as tissue growth and is primarily a function of milk intake (Totusek *et al.*, 1973, Black, 1982, Castells *et al.*, 2014, Bartle *et al.*, 1982, Montsma, 1960). Therefore clear variation in in growth within the first 3 days of life is likely explained by milk uptake. Additional evidence for higher milk consumption in the early group compared to the delayed group was calf plasma concentrations of bicarbonate, globulin and glucose. There were no experimental treatments involved in this study and it is not clear why these differences in milk uptake occur.

Differences in calf growth may be explained by delayed lactogenesis in the dams. Hartmann (1973) milked two quarters of each dairy cow daily from late pregnancy and daily milk yield from the quarters increased rapidly from <1 kg/d at day 2-3 pre-partum to approximately 12 kg/d 2-3 days post-partum. In the same cows, the remaining udder quarters of each cow were milked only from day of parturition and daily milk yield increased from 2.9±0.3 L/day to 8.6±1.4/day at day 3 post-partum. Given the short period between initiation of ample milk synthesis and parturition, the risk factors affecting milk yield may primarily be acting within the week pre-partum and during lactation itself. The important risk factors for first-week post-partum lactation yields in northern Australian beef herds are not clear.

Reports in dairy cattle describe how milk yield is reduced by cow nutritional deficiency (Coulon and Remon, 1991, Cowan *et al.*, 1980). In *Bos indicus* beef cows, studies focussed on lactation are limited, though Castells *et al.* (2014) reported that in tropically-adapted cows, phosphorus deficiency was associated with reduced early lactation milk yields. McBryde *et al.* (2013) reported that in Brahmans, each decrease in body condition (5-point scale) was associated with a reduction in daily milk yield of one kilogram. In Experiment 1, average body condition score for dams of delayed and rapid growth calves was 2.7±0.2 and 2.9±0.3 (on a 5 point scale), respectively (p=0.11). The delayed calves made up approximately half of the cohort and were only receiving maintenance levels of milk (Experiment 1). Therefore, if McBryde *et al.* (2013)'s association between cow body condition and milk yields holds for the class of animal in Experiment 1, then the delayed growth-type calves would be receiving a litre less than the daily requirement if the dams were in body condition score two. The mechanistic model demonstrated that calves

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neonatal mortality rate was calculated as percentage of total foetal and calf mortalities that occur during the first week of life. Holroyd also reported a pre-natal loss of 3.5% of pregnancies.

consuming increasing volumes of milk through neonatal life that averaged 3.8±1.4 L/12 hours and 2.6±0.7/12 hours, predicted neonatal survival and mortality, respectively. Reynolds *et al.* (1978) reported that Brahmans produce 2.8 kg per 16 hours and McBryde *et al.* (2013) reported that Brahmans cows produced 3.3 kg per 24 hours. If these reports for milk yield are representative of that occurring across breeding herds of northern Australia, then reductions in cow body condition may be leading to reduced milk yields and neonatal dehydration mediated mortality.

In Experiment 2, the incidence of neonatal calf mortality for dams with body condition score ≤2 and >2 was 3.7% (0.77% - 10.44%) and 2.84% (1.85% - 4.17%) respectively (p=0.5). In Experiment2, approximately 25% of cows were in body condition score ≤2 at calving.Vargas *et al.* (1999) reported that within second parity Brahman cows producing a live calf, those with body condition 3/5 had a lower weaning rate than those in body condition 4 and 5. McGowan et al. (2014) demonstrated that for tropically-adapted cows in low-phosphorus situations, a decrease in foetal and calf mortality of 3% is associated with maintaining cows above condition score of 3/5. Given the above reports, if the percentage of Experiment 2 cows in ≤2 body condition at calving was higher than 25%, milk delivery and incidence of neonatal mortality may have been be higher. It is not clear if the effects of low dam body condition on calf mortality are mediated by reduced dam milk yeilds or poor nourishment of calves during foetal development that leads to a reduction in abiltiy to suckle. Direct measurement of milk yields and measures of nutritional status, including body condition and phosphorus should be measured if possible in future studies on calf mortality.

In dairy cows, heat stress in late gestation is associated with reduced mammary development (Tao *et al.*, 2011) and heat stress during lactation itself results in a lag effect of reduced milk yield 24-48 hours later (Collier *et al.*, 1981, West *et al.*, 2003). In Brahman cows, there is an apparently-linear decline in milk yield as ambient temperature increases from 35^{o}C to 43^{o}C (Brody, 1956). This increase in temperature appeared to explain a drop in milk production of approximately 1 kg/day. A temperature-humidity index >79 for >14 days consecutive around calving is associated with increased foetal and calf mortality (McGowan *et al.*, 2014).In Experiment 2, the longest duration of temperature-humidity index >79 during pregnancy was six days. Within Experiment 2 calves, there was a low frequency of weak newborns (2%) and low incidences of neonatal mortality (4.7% in the northern forest and 3.1% in the southern forest), which also indicates low frequency of neonatal dehydration. Experiment 2 therefore does not disqualify the hypothesis that prolonged heat stress is associated with calf mortality. Further study is required to clarify the specific mechanism driving the association between prolonged high temperature-humidity index and foetal and calf mortality. The specific mechanism may be related to reduced dam lactation or calf vigour and therefore calf dehydration. In addition, calf rate of dehydration is exacerbated in with high ambient temperature (Fordyce *et al.*, 2015). Other possible mechanisms explaining the association

may include higher rainfall after a dry spell and therefore boggy conditions for calves or reduced efficiency of pasture growth (Bastin *et al.*, 2014, Hall *et al.*, 1998).

Variation in milk uptake and therefore hydration status may also be affected by calf ability to suckle. It is critical that the calf is able to access milk within the first days of life for adequate hydration (Fordyce *et al.*, 2015) and passive immunity protection (Weaver *et al.*, 2000). In Brahman calves, poor birth vigour has been associated with neonatal mortality (Muller, 2007). This is consistent with the report of Kim et al (1988), where Brahman calves without an instinct or ability to suckle took 409 minutes from birth until suckling, while other Brahman calves took 227 minutes. In Friesian calves bottle-fed their first colostrum *ad*-libitum, uptake volume was associated with calf vigour, as defined by attempting to stand within the first hour of life (Vasseur *et al.*, 2009). Therefore it is likely that the variation in ability for Brahman calves to suckle is associated with their milk uptake volume within the first days of life. Further, ability to suckle during neonatal life is highly likely to result in dehydration mediated mortality (Fordyce *et al.*, 2015).

Not all weak calves are able to stand and those that do can be apparently insensitive to teats during teat-seeking behaviour (Kim *et al.*, 1988), which is consistent with reports of calf autopsies where hairballs have been found in the calf abomasum, derived from hair around the udder or from the calf licking itself (Bellows *et al.*, 1987). The risk factors for calf inability to suckle are not clear for northern Australian beef breeding herds. Delayed- and rapid-growth calves in Experiment 1 had respective birth weights of 30.2±1.2 kg and 29.6±1.5 kg ($p=0.6$). These results indicate that the difference in growth type groups may be more likely due to differences in cow lactation than calf ability to suckle, based on reports describing how reduced birth weight is associated with increased frequency of poor birth vigour and mortality within a month of birth(Schmidek *et al.*, 2013).Despite the possibility that compensatory gain in birth weight can be made through foetal growth, stress on the conceptus critical stages of development could have 'all or none' type of effect(Funston *et al.*, 2010, Hubbert, 1973). For example, uterine insufficiency during critical stages of neuronal differentiation may lead to birth of calves that are insensitive to teat seeking. Reports in other species describe how foetal programming may impact on post-partum physiological parameters independent of birth weight. For example, nutritional deficiency at peri-embryo-implantation in sheep was associated with reduced brain weight and possibly compromised cardiovascular control for the progeny at one year of age (Gardner *et al.*, 2004). The lambs in both study groups had similar birth weights (Gardner *et al.*, 2004). Therefore low calf sensitivity in teat-seeking in tropically-adapted calves that occurs independent of birth weight (Kim *et al.*, 1988) may be explained by insult to the early conceptus with effects that may ultimately lead to reduced neural or cardiovascular development (Gardner *et al.*, 2004) by neonatal life.

Despite some weak calves being born of normal birth weight, in Brahman calves, compared to calves of moderate birth weight, those of low birth weight were at higher risk of mortality (Reynolds et al 1980). This is consistent with a study where in a dry-tropical environment, Brahman calves that died during neonatal life were on average 6.1 kg lighter than those that survived (Muller, 2007). Yates *et al.* (2012) reported in their review that heat stress on the cow during gestation may lead to placental insufficiency, restricted foetal nourishment and consequently the birth of low-birth-weight, low-vigour calves. Bull *et al.* (1974) reported that for a herd of *Bos taurus* cows, those on low and high levels of protein during the last 60 days of gestation, the percentage of weak calves born was 15% and 0%, respectively. Nutrition during gestation has also been associated with birth weight (Micke *et al.*, 2010, Fordyce *et al.*, 1993). In Experiment1, calf vigour score did not differ between delayed and early growth groups (p=1.00).In Experiment2, incidence of neonatal mortality for calves of birth vigour <3 and ≥3 were 76.2% (52.8 - 97.8) and 1.3% (0.7 - 2.2), with a risk ratio of 60.4 (29.8 - 122.6; p≥001). Incidence of neonatal mortality for calves of birth weight ≤28 kg and > 28 kg was 7.56% (4.09% - 12.58%) and 2.16% (1.26% - 3.43%), respectively. The incidence of deaths in the lighter calves was 3.45 (1.73 - 7.11; p<0.001) times higher than heavier calves. Therefore there is very high risk of neonatal mortality in weak calves. The ≤28 kg threshold in Experiment 2 was the lowest 20% percentile of birth weight. In the study of Bunter *et al.* (2013), calves below the 20th percentile for birth weight had a 2.12 times higher odds of experiencing mortality than calves of higher birth weight. The percentage of weak calves was low (18%) in Experiment2. This is consistent with the very low percentage of cows in poor condition at calving, given that protein deficiency through gestation may reduce cow body condition (Menges, 1994) and the known effect of protein deficiency in reducing the vigour of newborn calves (Bull *et al.*, 1974). In northern Australia, low protein nutrition for the dam from mid-gestation to around calving was associated with high foetal and calf mortality (McGowan *et al.*, 2014), which may at least partially explain the impact of dam nutrition on neonatal calf vigour.

Heat stress may reduce blood flow to the foetus (Reynolds *et al.*, 1985), which may in turn reduce vigour of newborns. Edwards (1982) reported that time of birth in the calving season may at least partially explain vigour to stand and suckle, an apparent function of ambient temperature. In addition, a study in Brahmans by Wythe (1970) showed that incidence of low birth vigour varied from 3.5% to 11.8% over a number of years, and that birth vigour was impacted significantly by sequence of birth in the calving season, temperature at birth and precipitation at birth. This study was conducted in Texas, USA, where the hottest calving month was in July with an average temperature of 28^{o}C and the coolest calving month was in January, with an average temperature of 11^{o}C. Month of birth effects may involve both environmental and nutritional risk factors, though nutrition was not investigated in Wythe's study.

In Holstein cows, calf birth weight was lower when cows were not shaded during the third trimester (Collier *et al.*, 1982). Foetal weights at day ~130 of gestation in pregnant ewes exposed to 0, 55 and 80 days of heat stress from day 35 of gestation were 3391±331.2, 1841±30.6 and 882±141.9 g, respectively (Galan *et al.*, 1999). Therefore impacts of heat stress on birth weight are likely impacting on foetal growth in a cumulative manner, as opposed to having permanent effect at a specific stage of gestation (Galan *et al.*, 1999). Impacts of heat stress at particular points in conceptus development may not be able to be compensated by relief from heat stress in other stages of pregnancy, for example where heat stress in early conceptus development may have an effect, similar to the effects that occur from dam nutritional deficiency during early conceptus development (Funston *et al.*, 2010). This is supported by the study of Biggers *et al.* (1986), where *Bos taurus* cows were subjected to differing day-time heat, temperature and relative humidity conditions (37±1^{o}C and 27±2% vs.21±1^{o}C and 35±10%) during approximately 7-15 days after conception, high heat stress was associated with a reduction in conceptus weight, as measured at approximately 16 days after conception (72.8 vs. 157.9 mg). In Experiment 2, though temperature-humidity index was not consistently high to for the length of time that has been associated with increase foetal and calf mortality (McGowan *et al.*, 2014), a peak of temperature-humidity index occurred during early gestation. Therefore, in situations where temperature-humidity index is high for a prolonged period, at least part of the effects of high temperature on foetal and calf mortality may be explained by impacts of early conceptus programming.

Although this thesis does not suggest specific management interventions that producers can use to reduce calf mortality, it does indicate that future calf mortality research in northern Australia should focus risk factors that may be alleviated or eliminated by producers to reduce their incidence of on dehydration mediated calf mortality.

6.1 Conclusion

In a small cohort of calving Brahman cows, approximately half of their calves were only receiving sufficient milk to maintain weight during the first 2-4 days of life, while the other calves were receiving enough milk to gain weight rapidly from birth, leaving the former at risk of calf mortality under adverse conditions. For 683 cows in the southern forest and 297 cows in the northern forest, there were low frequencies of nutritional and environmental stress associated with low respective incidences of neonatal mortality of 3.1% and 4.7%. Therefore low calf mortality may be achieved in any country type, provided that a low proportion of calves are exposed to high risk for mortality. Given the low proportion of cows in poor body condition and of neonatal mortality, nutritional management limited the opportunity for dehydration-mediated neonatal mortality. A mechanistic model that synthesises data from previous studies assisted with

understanding how low milk uptake through neonatal life may ultimately lead to dehydration mediated mortality. These studies supported the hypothesis that insufficient milk delivery may be a major reason for elevated calf mortality under adverse environmental conditions.

7.0 References

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